Neuroimaging markers of the delayed effects of ketamine

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NEUROIMAGING MARKERS OF THE DELAYED EFFECTS OF KETAMINE

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A thesis submitted to King's College London for the degree of Doctor of Philosophy for Neuroimaging 2019

Abstract

Major Depressive Disorder is a chronic mood disorder, affecting approximately 320 million people worldwide. Rumination associated with biased AM recall and anhedonia linked to altered reward processing, are two of the most debilitating symptoms of depression that could persist in remission and are not successfully targeted by commonly prescribed antidepressants. Ketamine, a glutamate receptor antagonist, has emerged in the last two decades as a potent antidepressant with robust effects. The drug's fast acting (2h post drug administration) and relatively long-lived antidepressant action (24h-48h after a single ketamine infusion) also targets anhedonia and rumination. (Lehman et.,al 2016, Lally et al., 2014) However, the mechanism by which the drug exerts its antidepressant effects in the brain are not well understood.

The aim of the present study was to identify brain areas that present with significantly altered activations 2h after the ketamine administration, when the antidepressant effects of the drug become detectable, and are important for reward processing and emotionally valenced AM (Autobiographical Memory) recall. For that purpose, we recruited remitted depressed volunteers who were scanned while performing an AM task (VAMP) and a reward processing task (MID). We hypothesized that ketamine, during the MID task would significantly increase striatal activations while during the VAMP task, the drug would modulate activations in the PCC, the sgACC and the amygdala, compared to placebo. These brain areas could serve as neuroimaging markers that could be linked to ketamine's antidepressant action.

For this study we recruited 37 remitted-depressed, drug-naïve, male and female volunteers who took part in a double-bind, placebo-controlled, cross-over design. Ketamine (0.5mg/kg) and placebo ware administered intravenously over a 45min continuous infusion. Two hours after the drug administration, participants were scanned while performing the MID and the VAMP task. The MID is a reaction time task during which participants need to perform fast button presses, in response to a target stimulus, to win different monetary rewards (Knutson et al., 2001). A cue that is presented before the target signifies the value of the reward that they could win in each trail. The reward anticipation and feedback phases of this task were analysed at a whole brain level and activations were also examined at predefined ROIs associated with reward processing, namely the striatal regions, the VTA, the amygdala and the insula.

The VAMP task is a personalised AM task that was developed specifically for this study. During the task statements from positive, negative and neutral events are presented to participants who are asked to recall those events and rate their emotional valence at the time of the scan. The fMRI data from this task were analysed at a whole brain level and a task connectivity analysis was performed for predefined ROIs that are associated with emotional memory recall and present with altered activations in depression, namely the amygdala, the PCC and the sg ACC.

Scales and questionnaires that measure rumination, anhedonia, subjective well-being and the dissociative/psychotomimetic effects of ketamine were administered at appropriate time points during the study.

Ketamine 2h after its administration did not produce any significant changes to the anhedonia and the subjective well-being of our participants. Whole brain analysis of the MID task did not reveal any significant changes during the anticipation and feedback phase of the task, 2h post ketamine, compared to placebo. The ROI analysis of the task revealed significant increases in the activation of the VS (Ventral Striatum) and the caudate. These increases were identified during the feedback phase of successful and unsuccessful MID trials that were associated with low monetary rewards. Also activity in the VTA (Ventral Tegmental Area) increased during the feedback phase of unsuccessful trials, irrespective of the reward magnitude.

Whole brain analysis of the VAMP placebo data revealed that the precuneus, the middle frontal gyrus and the thalamus presented with significantly altered activation during active AM recall. Ketamine, 2h after its administration and at the whole brain level, did not produce any significant changes to the task, compared to placebo. When the task connectivity was examined between ketamine and placebo, ketamine decreased connectivity between the amygdala and the visual cortex for positive and negative memory recall, compared to neutral. Decreased connectivity between the amygdala and the hippocampus was also identified for positive memory recall compared to neutral, when ketamine was compared to placebo.

Ketamine, 2h after its administration, when its concentration in the blood is very low, significantly altered activations in brain areas that are important for anhedonia and emotionally valenced AM recall. The increases in the activation of the VS and the caudate during low in reward magnitude trials, could be linked to the participants' increased sensitivity to these trials under ketamine, compared to placebo. This effect would be relevant to the drug's antidepressant action which could have a positive impact on motivational processes engaged during the MID.

The decreased connectivity that was identified after ketamine between the amygdala and the visual cortex, could be associated with ketamine reducing the visual imagery that is associated with emotionally valenced AM recall. This reduction in the connectivity occurred during positive and negative memory recall, compared to neutral and could be associated with reconsolidation processes that might alter the emotional valence of the recalled memory. This would suggest that ketamine as an antidepressant could have beneficial effects on maladaptive rumination.

Statement of work

The present research study of ketamine's delayed effects in remitted depressed volunteers was funded by Johnson and Johnson via a research grant to King's College London. My tuition fees and stipend were covered via a scholarship from the BRC (Biomedical Research Centre).

I was involved in all the stages of ethics approval, volunteer recruitment, data collection and analysis. Specifically, I contributed to the ethics application, amendments and extensions in collaboration with my supervisor Prof Mitul Mehta. I was solely responsible for the recruitment of participants, the data collection and the analysis.

For the analysis of the results and the write up of this thesis I have received guidance and feedback from my supervisor Prof Mitul Mehta.

Preliminary results of this research have been presented to British Association of Psychopharmacology Meeting (2019) as well as the CINP (International College of Neuropsychopharmacology) thematic meeting (2017).

Acknowledgments

This thesis would have never been possible without the genuine interest and selfless contribution of my amazing volunteers, the constant support of our research team and the trust of our funders. Steph and Ndaba, thank you for making every study day unique and enjoyable. I am very grateful to you both since with your experience and genuine interest for the well-being of the participants made me, and most importantly them, feel comfortable and safe!

The recruitment for this study was not easy and Dr Declan Jones with Dr Maura Furey were extremely patient, supportive and encouraging throughout that process. Thank you!

A very special thank you to my friends! Barbara, Daniel, Ottavia and Panos (in alphabetical order to avoid ruining our friendship…) you are my family away from home and you have given me the most joyful memories! I hope we will share many more beautiful moments together!

The four years of this PhD research would have been very difficult without the support of my office mate! Pete, thank you for sharing ideas with me, discussing my problems, supporting me during tough times. You are an excellent colleague and a very dear friend!

A very special thank you to Dr Carlos Zarate and Dr Argyris Stringaris who gave me a lot to look forward to during the last tough months of writing-up!

Last but not least, I would like to extend a very special and heartfelt thank you to my supervisor, Prof Mitul Mehta. Mitul, thank you so much for your trust in me. Your guidance and your support have been constant and unwavering throughout the six years that we have worked together. Your enthusiasm and genuine interest for research are indeed inspirational. I am looking forward to collaborating more with you in the future. Thank you!

Finally, a very special thank you to my little niece Filothei-Thalia. My dearest Thalia, your love and your laughter have emotionally nurtured me and supported me in all my days since I first met you. You are my inspiration! When close to you I feel able and strong. Watching you grow is perhaps the most amazing and exciting research project of my life!

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Introduction

Depression

1 General characteristics with focus on rumination, biased AM recall and anhedonia

Major depression is a common illness that severely limits psychosocial functioning and diminishes quality of life. In 2008, WHO (World Health Organisation) ranked major depression as the third cause of burden of disease worldwide and projected that the disease will rank first by 2030. The most probable period for onset of depression extends from mid-adolescence to mid-40s, but 40% of individuals experience an episode before they reach 20 years of age. Across the lifespan, depression is as prevalent in men as in women (Malhi and Mann, 2018). Depressed mood and anhedonia are considered the fundamental symptoms of the illness with neurocognitive as well as neurovegetative symptoms also present.

Overgeneral memory, specifically in relation to recall of autobiographical memories, and rumination are also amongst the most common cognitive characteristics of depression (Hamlat et al., 2015 Williams, 2006). Although they are not considered necessary for a formal diagnosis of the illness, these cognitive symptoms appear to play a central role for the initiation and maintenance of a depressive episode (Williams, 2006). These two cognitive characteristics are also key points of focus for several theoretical models which have been developed in order to link dysfunctional cognitive processing with depressive symptoms (Disner et al., 2011, Williams, 2006, Dalgleish and Werner-Seidler, 2014). Overgeneral AM recall along with a bias towards easier negative memory retrieval and increased rumination could provide a neuropsychological basis for a better understanding of depression (Fivush, 2011).

2 Depression and Autobiographical Memory

2.1 Autobiographical Memory definition. Encoding and recall of autobiographical memories

AM (Autobiographical Memory) encompasses our recollection of specific, personal events and it is very important for the individual's sense of self. It is important to ensure self- coherence as well as to guide individuals towards goal directed activities (Rubin et al., 2003). AM has been broadly conceptualized to be divided into personal semantic information (facts about one's self) and personal episodic information (unique events) (Tulving, 1972). One striking feature of this type of memory is that for each event different levels of detail specificity are being contained (Williams et al., 2007).

The recall of AMs appears to be a multi-level process that becomes dysfunctional during several affective disorders (Kohler et al., 2015). Generally, when recalled, AMs have specific characteristics that play a very important role in the understanding of how these memories are encoded and consequently retrieved (Williams et al., 2007). Although they can vary in their level of specificity, AMs are organized into coherent narratives enriched with contextual details and when retrieved, these memories are usually accompanied by a feeling of vividness and rich sensory and perceptual details (Conway, 2005, Conway and Pleydell-Pearce, 2000).

Generative and directive recall seem to be the two main pathways for retrieval of a specific AM and were first described by Norman and Bobrow in 1979. Generative recall is usually activated by a particular "cue" and the process involves the initial accessing of AMs in a general- categorical- level. As the process develops, more specific AMs are being retrieved which are relevant to the specific cue. The process of generative recall concludes when a specific memory is recalled. Direct retrieval refers to the spontaneous activation of an AM. This manner of recall bypasses the general stage of non -specific/categorical recall and leads to an immediate recollection of a specific event (Conway and Pleydell-Pearce, 2000). Although both generative and direct retrieval are equally effective mechanisms for the recollection of AMs, the former is a more controlled process which allows different levels of specificity during memory retrieval depending on which stage of generative recall this process is aborted (Holland and Kensinger, 2010). Termination of this process at the very general – categorical – level of recall has been linked to overgeneral memory and is important for depression (Warne et al., 2019).

Despite the manner by which they are recalled, the quality of AMs seems to be closely related to the way they are encoded and maintained. The maintenance of a memory is not a static process but rather one that continues in the hours, days and years following an experience (Kim, 2019). Several factors of the experience itself would influence the way AMs can be assembled, retained and afterwards retrieved (Fivush, 2011). The arousal and consequentiality of the event as well as its emotional valence seem to be among the key factors that could influence the quality of AMs (Holland and Kensinger, 2010).

The presence of arousal appears to trigger a cascade of neurochemical interactions, the end result of which would be the formation of a particularly durable memory. Events related to strong personal involvement elicit physiological responses such as the release of glucose and adrenal hormones into the blood stream that often result to strongly encoded memories (McGaugh, 2004). These cumulative effects of emotion would also influence the consolidation phases of the memory and would increase the person's ability to remember that event. Moreover, events with high personal involvement or self-relevance, concepts closely tied to the idea of consequentiality are more likely to be remembered than events with less personal relevance (Fivush et al., 2011, Sharot et al., 2007). When information is encoded in a self-referential fashion, distinct mechanisms are engaged which boost the strength of the encoded memory and make it more resilient to forgetting (Klein, 2012).

Beyond arousal and consequentiality, the valence of an experience could also influence the likelihood of remembering an AM as well as the accuracy with which each event can be remembered (Kim et al., 2018, Wisco and Nolen-Hoeksema, 2010). In several studies that have looked at healthy volunteers, positive events seem to come to mind more readily than negative ones. Some of this mnemonic benefit for positive events may stem from the fact that positive experiences are more likely to be encoded as strongly self-relevant and may also be more easily integrated into a person's conception of themselves that could thus lead to a facilitated retrieval of that event. Although negative memories could also be associated with high-arousal, negative emotions such as fear or anger, the way however, that these negative feelings could shape the manner in which negative experiences are been encoded and retrieved might differ to that of positive memories (Macleod et al., 1994, Teasdale et al., 1980).

The bias towards positive memory recall which is detected in healthy volunteers appears to be reversed in depressed individuals who tend to recall more negative memories (Williams et al., 2007). Whether this bias towards negative memory recall is due to deficits in the process underlying positive memory retrieval or whether negative memory recall is facilitated due to psychological processing remains a debate. In order to be able to resolve this issue we need to examine the brain areas that are important for AM recall and how their function is altered in depression or could be influenced by psychological factors such as mood. In the section that follows we will try to investigate the role of some of the most prominent brain areas for AM recall, namely the medial temporal lobe, cortical brain areas and the amygdala. In order to understand the role of those brain region in the formation and recall of AMs, evidence would be drawn from post-mortem studies as well as neuroimaging research.

2.2 The Autobiographical Memory recall network of brain regions

The medial temporal lobe is one of the key brain areas that seem to have a central role in AM processing. The important role of that area in AM encoding has already been highlighted in Alzheimer's disease where patients with medial temporal lobe damage are unable to register life events into long-term memory (Buchanan et al., 2006, Buchanan et al., 2005). In fact, the medial temporal lobe, and the hippocampal complex more specifically, is referred to as a hub for the AM system (Greenberg et al., 2005). Damage to the hippocampal complex does not prevent information from being processed but rather it prevents the organised binding and indexing of future events (Ryan et al., 2001). Hippocampal damage therefor, results in anterograde amnesia by preventing new information from becoming bound together in a fashion that allows them to be retrieved. It also results in retrograde amnesia because the damage destroys the indices that would have pointed to components of past experiences (Fournier et al., 2019) thus preventing their retrieval.

The medial temporal lobe, however, does not act alone to encode and/or retrieve autobiographical experiences. Research has revealed that memories are not stored within a single area of the brain. A distributed network of activity throughout the cortex seems to be implicated in mnemonic processing (Lin et al., 2016, Desalaar et al., 2008, Kohler et al., 1998). Indeed, research has revealed, that memories are not stored within a single area of the brain. There is evidence indicating that during AM retrieval all the brain regions that have been activated during the encoding in memory of a specific event (Kohler et al., 1998, Wheeler and Buckner, 2004, Kahn et al., 2004) are also active. Moreover, the specific regions that are

activated during AM recall and the magnitude of activity within those regions could vary according to the level of details that are remembered (Richter et al., 2016). Commonly activated regions during AM recall include the medial and lateral prefrontal cortex, the medial and lateral parietal cortex, the amygdala and sensory cortices within the occipital and temporal lobes (Wheeler et al., 2006, Nawa and Ando, 2019). Among those areas the amygdala and their role in AM has been significantly studied.

Research indicates that the role of the amygdala in AM is rather complex. There is evidence indicating that when amygdala activity is strong, during encoding of emotional events, these events are more likely to be remembered (Dolcos et al., 2012, Hamann, 2009). This could suggest an interplay between the strength of the encoding, the emotional valence of the memory and amygdala activity. However, amygdala activity does not lead to enhanced memory of all the details of that emotional experience. Engagement of the amygdala would boost the retention of some event details (Kensinger and Schacter, 2005), have no effect on the retention of other details (Dougal and Rotello, 2007, Onoda et al., 2009) and even sometimes impair memory for other types of information (Strange et al., 2003).

Despite the mixed relations between amygdala activity and subsequent memory of the details for a specific event, amygdala activity during encoding seems to correlate with the emotional quality of recall. AMs that have been linked to enhanced amygdala activation during their encoding appear more vivid when recalled and individuals seem to be highly confident as to the accuracy of these AMs (Mickley and Kensinger, 2008). The reason of this strong connection between amygdala activation and the emotional quality and accuracy of AMs remains a topic of ongoing investigation. A possible suggestion could be that individuals ascribe vividness and confidence on the basis of their memory for only a subset of details, so that by enhancing memory for select details, amygdala activity may also be enhancing the confidence or vividness with which people will later re-experience an emotional event (Phelps and Sharot, 2008). Although a lot of research has focused on the amygdala, as we mentioned before, that brain area does not act in isolation but is part of a larger network of bran regions that play a role in AM recall.

A particularly interesting interplay takes place between two brain circuits that are activated only when individuals have personal involvement in the AMs (Lindquist et al., 2012). These two brain circuits are the one connecting the left hippocampus to the medial prefrontal cortex and another linking the medial prefrontal cortex to the right amygdala. They both appear to be in synchrony only during recall of events in which the individuals had

been directly involved (Muscatell et al., 2010). The hippocampus is thought to support the retrieval of AMs with rich contextual detail thus, the activation of the hippocampus corresponds with the ability to remember the spatial and temporal context in which life experiences occurred (Maguire, 2001, Svoboda et al., 2006). By contrast, amygdala engagement during retrieval does not seem to correspond with retrieval of these type of contextual details but instead seems to reflect the processing of affectively rich, internal information (Buchanan, 2007). These systems, of perceptual and conceptual retrieval, respectively, may work together to integrate internal and external details, but only when information is high in personal involvement (Sheldon et al., 2019).

The interaction between the amygdala and the hippocampus is not only important for memory retrieval but also for memory consolidation (Nieuwenhuis and Takashima, 2011). Memory consolidation is a difficult process to isolate mostly because it refers to a series of processes that occur over time and thus, it is very hard to capture a snapshot of a point when consolidation has occurred. Most of the research on memory consolidation seems to be focusing on sleep. In a study where participants were asked to retrieve negative emotional and non-emotional information after a 12- hour delay that either induced a night of sleep or a day spent awake, the hippocampus was activated during successful retrieval of negative memories regardless of whether participants have slept or not (Payne and Kensinger, 2011). However, sleep led to a shift away from a diffuse memory network (including lateral prefrontal and parietal cortices) and toward a more refined and integrated network of limbic regions (consisting of the amygdala and ventromedial prefrontal cortex) (Nieuwenhuis and Takashima, 2011, Payne and Kensinger, 2011).

The aforementioned findings indicate that the hippocampus has a broader role during AM recall, which seems to be independent of reconsolidation processes. The amygdala on the other hand seem to be involved in the fine tuning of recall by controlling aspects such as the vividness of the AM as well as the level of detail of those memories (Kensinger, 2009). Apart from the temporal regions and the amygdala, other brain areas such as the OFC (orbitofrontal cortex) (LaBar and Cabeza, 2006) and occipital areas (Vuilleumier and Driver, 2007) also appear to involved in AM encoding and retrieval. All those brain regions and their implication in AM formation, however, have been studied in healthy volunteers. Patients with depression present with AM deficits that could alter the characteristics of their AM recall and could be the result of differential activations in these brain areas.

2.3 Biased recall of Autobiographical Memories in depression

2.3.1 Overgeneral memory

Individuals with MDD demonstrate a tendency to retrieve fewer specific and more categorical AMs compared to healthy controls (Kohler et al., 2015). Interest in overgeneral memory has been fostered by findings suggesting that this phenomenon could be closely associated, and might have a causal link with, other important aspects of physiological functioning which are affected in depression. Specifically, overgeneral memory has been associated with a. impaired problem-solving (Raes et al., 2005, Goddard et al., 1996) b. difficulties in imagining future events (Williams et al., 1996) as well as c. delayed recovery from episodes of affective disorders (Dalgleish et al., 2001, Peeters et al., 2002). The phenomenon of overgeneral memory has also been deemed important because memory remains overgeneral in those with a history of emotional disorder, even if not currently in an episode (Mackinger et al., 2000). This is significant since it means that overgeneral memory can be observed without needing to be activated by low mood and might therefore act as a before or between episode marker of future vulnerability to depression.

Several cognitive models have been developed in order to explain overgeneral memory recall in depression. As mentioned earlier, according to the Conway and Pearce model, retrieval of AMs could be either generative or directed (Conway and Pleydell-Pearce, 2000). Overgeneral memory arises when individuals truncate their search during generative, top-down retrieval at too high a level, when only general descriptive information could be assessed (Ros et al., 2018). Generative retrieval is usually activated by specific cues which allow the individual to access memories at a very general- categorical level- and then, more specific memories that are linked to the specific cue, are retrieved and evaluated in order to finally recreate a specific AM (Williams et al., 2007).

According to the Conway and Pearce model, depressed individuals seem to suffer from a "mnemonic interlock" which maintains recall of autobiographical memories at a general event-knowledge level. This retrieval strategy could potentially have a protective role for the individual. If the retrieval mechanisms were to proceed further the individual risks to access among other- intense negative memories- which could worsen someone's mood or trigger ruminative processes. As a result, depressed and suicidal patients abort memory searches for a specific event prematurely, when only the general description stage of a particular autobiographical event has been reached (Norman and Bobrow, 1979). This truncated search is called a "dysfacilitation" of the retrieval process and is assumed to underlie the retrieval of overgeneral memories (Dearing and Gotlib, 2009, Gillihan et al., 2007).

2.3.2 A bias towards negative memory recall

A few studies have suggested that depression might be characterized by reduced positive processing, which could demonstrate as a tendency to recall more negative than positive AMs, rather than increased negative processing (Kohler et al., 2015). This supports the idea of an "optimism bias" which could indicate that mentally healthy people are unrealistically positive thus "positive bias" reduces as depressive symptoms emerge. Although depressed individuals might appear as more negative, it is unclear whether this is because they are more likely to attend to or remember negative information or less likely to attend to or remember positive information or even whether both are occurring (Lewis et al., 2017). It is also unclear whether the alterations in negative and/or positive processing precede or follow depressive symptoms.

One of the key questions that the difficulty in positive AM recall raises is whether this difficulty manifests as a general change of brain function and/or anatomy that would impair the recall of all AMs or whether it is specific to positive memories. If AM recall engages the same set of brain areas irrespective of the special characteristics of the memory (alertness, valence and consequentiality) the issue of impaired negative and neutral memory recall in depression should also be raised.

3 Depression and Rumination

3.1 Rumination definition

Rumination is an emotionally evocative experience, during which an individual repeatedly thinks about past experiences, focusing on the feelings, meanings and consequences of those events (Schafer et al., 2017). Rumination is a central process for depression as well as a well-known risk factor for developing MDD especially during mid-life (Bromberger et al., 2015). The intensity of ruminative thinking could also predict the onset of new episodes and the severity and duration of existing depressive episodes(Abela and Hankin, 2011). Brooding and reflection are the two aspects of rumination (Nolen-Hoeksema et al., 2008). Brooding involves the tendency to dwell on negative consequences of one's low mood (Treynor et al., 2003). Reflective pondering involves attempts to understand the reasons for one's depression. Brooding is considered to be the most maladaptive of the two aspects of rumination and has mainly been implicated in mental health problems (Luca, 2019).

3.2 Brain areas and networks that are important for rumination

Although intense ruminative thinking has been associated with low mood and depression, rumination is a thought process that occurs to healthy individuals as well (Watkins, 2008). Several studies, using neuroimaging have tried to identify the brain areas that are activated while ruminating. Intense self-focus but also retrieval and reconsolidation of AMs are involved during ruminative thinking and as we have already mentioned before, these processes could be highly influenced by the mood state of the individual(Thomsen, 2006). Rumination is a very difficult process to study with fMRI (Ferdek et al., 2016). It requires intense focus, which is difficultly achieved in the noisy scanning environment, moreover, it is very difficult to ensure that individuals remain focused for the duration of the scan. Another factor that should also be considered is that recalling past AMs could be stressful for individuals who engage in ruminative thought of especially negative memories.

The fMRI studies that examined rumination have mainly looked at brain connectivity as a whole rather than focusing on individual brain areas. These studies have revealed that the DMN (Default Mode Network) and specifically the PCC, which is considered the main hub of that network play an important role during rumination. The PCC along with the medial prefrontal cortex and angular gyri show increased activation during ruminative thinking (Hamilton et al., 2015). The DMN is a network that is activated when individuals are self-focused and not engaging on a specific task although studies have shown that its activity could also be task related (Smith et al., 2018). Some of the areas that form part of this network including temporal regions are also important for AM recall (Grieder et al., 2018). In depression, it is believed that the DMN appears to be overactive making it easier for depressed patients to remain focused on the recall of negative AMs, rumination thus becomes a very relevant process for depression (Sheline et al., 2009, Lehmann et al., 2016).

Rumination is also one of the key factors that have been linked to cognitive vulnerability in depression. It has been suggested that rumination could act as a potent mediator between stressors and prolonged stress-related processes (Michl et al., 2013). Specifically, ruminative thinking could facilitate the mental representation of specific stressors that would contribute to negative mood and prolong depressive symptoms (Watkins, 2008). The interplay between rumination, AM recall and depression has been in the core of several theoretical cognitive models that try to explain the initiation of a depressive episode and the prolongation of depressive mood. Here, we will discuss the most prominent of these models that have mostly influenced the design and hypotheses of research studies.

4 Autobiographical Memory, Rumination and Depressive Symptoms

4.1 The CaR-FA-X model

The CaR- FA-X model was first proposed by Williams in 2006 and it owns its name to the three key processes that could lead to overgeneral memory recall and depression: Capture and Rumination, Functional Avoidance and finally eXecutive control (Williams, 2006).

Capture and Rumination occur when ruminative processes are activated and are accompanied by disrupted retrieval processes. These dysfunctional retrieval processes include the potential facilitation in the recall of negative AMs as well as truncated generative AM recall. More specifically, the model suggests that difficulties accessing specific AMs could result from the Capture (Ca) of memory search efforts by consolidated categorical themes which are prone to cause depression-depressogenic themes (Barnhofer et al., 2002). These depressogenic themes could, in their own turn, engage analytical and evaluative brooding rumination. Brooding rumination often appears maladaptive and could further contribute to depressive mood (Luca, 2019).

The capture mechanisms could further be exacerbated by an ingrained Functional Avoidance (FA) that characterises depressed individuals as well as those in high risk of depression (Hallford et al., 2018). Functional avoidance is a means of regulating negative affect by avoiding autobiographical content associated with distress. Depressed individuals tent to actively avoid retrieving specific details of distressing autobiographical events which as mentioned before, traps the AM recall at a categorical level where whole groups of AMs are accessed but none of them specifically recalled (Barnhofer et al., 2002). The ability to counteract these dysfunctional processing mechanisms is further compromised by the limited eXecutive control (X) which is a consistent feature of depression (Watkins and Brown, 2002).

EXecutive control (X) refers to broad range of cognitive strategies used to manage, focus on, plan and carry out tasks (Roberts, 1996). Executive function is a product of executive control. Deficits in executive control are hypothesised to interfere with the retrieval of specific AMs because of the need to hold information in mind (updating working memory) and ignore irrelevant information. The CaR-FA-X model mainly tries to interpret overgeneral AM recall in depression (Conway and Pleydell-Pearce, 2000) without particularly focusing on other aspect of the disorder. The cognitive processes however, that are discussed in this

model are central for the understanding of the disorder and this model has influenced many research hypotheses that investigate the cognitive aspects of depression.

4.2 The cognitive model of depression

Beck's cognitive model of depression was developed about 40 years ago and is based on evidence demonstrating that depressed individuals show deficits in attention, the processing of emotional stimuli, as well as biased rumination and recall of negative memories. The model integrates cognitive and neurobiological processes which could be undermined by genetic and/or environmental factors. When activated, these processes would initiate a negative cognitive bias which then persists due to the attenuated cognitive control of depressed individuals.

In this model, biased acquisition and processing of information has a primary role in the development and maintenance of depression since it could lead to the development of depressive schemas. Depressive schemas are characterised by negative self-referential beliefs and could be the outcome of adverse events that occur to an individual's life. These negative self-referential beliefs are further exacerbated due to the biased acquisition and processing of emotional information in depression. Environmental stressors could activate these schemas which when activated confer vulnerability for depression. Specific biases in attention and memory which result from inhibitory deficits, such as deficits in executive control, could further contribute to the development of a ruminative response that perpetuates negative thoughts about one's self, the world and the future. Beck's model of depression is considered one of the most complete models that explain the cognitive aspects of the illness, however, it offers no insight to the brain areas that could be important for the cognitive processes that are involved in the model and appear to be dysfunctional in depression (Beck and Bredemeier, 2016).

4.2.1 Biased attention, increased self-focus, emotion over-reactivity

In an effort to better understand the neurobiological mechanism that could be implicated in Beck's model of depression Disner et al. have tried to provide a link between Beck's model and neuroimaging findings (Disner et al., 2011). Specifically, the known differences that depressed individuals present with, concerning the activation of different brain areas, have been associated with the neuronal processes that are relevant to the model thus providing the neurobiological basis for Beck's theory.

Specifically, depressed patients show decreased activity in the right DLPFC (DorsoLateral PreFrontal Cortex), right VLPFC (VentroLateral PreFrontal Cortex) and right superior parietal cortex (Beevers et al., 2010, Fales et al., 2008, James, 2012, Passarotti et al., 2009). This could account for the biased attentional focus on a negatively valenced stimulus which could block out the processing of other more positive information. The biased attention to negative stimuli in individuals with depression could also be enhanced by their inability to disengage from this particular negative stimulus due to increased self-focus (Corbetta et al., 1998). Depressed individuals also present with greater ACC activation when successfully inhibiting attention to negative stimuli, suggesting that contrary to healthy individuals depressed patients require greater cognitive effort to divert attention away from negative stimuli (Drevets et al., 2008b).

Excessive amygdala reactivity in individuals with depression could provide the neurobiological basis for the increased emotional reactivity of these individuals. The overactivation of the amydagala persists even when an aversive stimulus is no longer present (Young et al., 2012, Young et al., 2016). Increased amygdala reactivity creates bottom-up signals which are processed in higher cortical areas and could alter the perceptions of the environment leading to persistent negative information processing. The neurological deficits that could underlie this altered environmental perception could be associated with the reduced cognitive control over the amygdala associated with aberrant activation of the bilateral DLPFC (Connolly et al., 2017).

A parallel neurological pathway that could also contribute to sustained processing of negative stimuli in individuals with depression is the thalamocortical pathway. This pathway plays an important role for the organisation and processing of environmental stimuli. Elements of this pathway include the thalamus, a brain region which distributes afferent signals in several other areas including the dorsal ACC (Sherman and Guillery, 2002). The dorsal ACC also receives input form the DLPFC and the subgenual cingulate cortex which integrates emotional feedback from the limbic system and projects to higher order cognitive structures (Ray and Zald, 2012). During a depressive episode, individuals show increased thalamic activity (Greicius et al., 2007). This increase in thalamic activity could be interpreted as a compensatory mechanism attempting to make up for the reduced functional connectivity between the medial thalamus and the dorsal ACC (Brown et al., 2017). The dorsal ACC exerts less inhibitory influence over the limbic system in depressed individuals and more depressive limbic feedback is able to be processed via bottom-up pathways through the sgACC upstream to higher order regions (Banks et al., 2007). This in its turn, could lead to increased processing of negative stimuli and an inability to shift attention to external distractors in depression.

4.2.2 Rumination and biased recall of negative autobiographical memories

Prolonged processing of emotional experiences in people with depression- a result of ruminative thought- is probably maintained by impaired top-down cognitive control over limbic areas which is generally associated with hypoactivation in the left DLPFC and VLPFC concurrent with rumination. More specifically, DLPFC and VLPFC hypoactivity are correlated with altered patterns of rostral ACC activity which is thought to contribute to rumination by facilitating the inhibition of positive information and impeding the inhibition of negative information (Koseki et al., 2013).

Furthermore, individuals with depression, recall with more ease negative AMs, compared to positive AMs. The neuronal correlates that underlie this bias towards the recall of negative information involve brain areas that could facilitate a stronger encoding of negative AMs. Stronger encoding of negative AMs could lead to an easier retrieval of those memories and that could be attributed to the hyperactivity of the right amygdala which has been associated with better encoding of negative stimuli, but not positive or neutral (Young et al., 2018).

During encoding of negative AMs, amygdala activity is correlated with increased hippocampus, caudate and putamen activity which in turn would facilitate recall of negative but not positive information (Young et al., 2016). Moreover, the MPFC (Medial Prefrontal Cortex) is hyperactive during recall of self-relevant happy events and hypoactive during recall of self-relevant sad events in individuals with depression (Disner et al., 2011). As a result, depression requires greater cognitive effort – mediated by the MPFC -to recall happy personal memories whereas recall of negative memories requires less top down influence.

Taken together, these finding indicate that there is indeed a neurobiological basis for the cognitive model of depression as well as the CaR-FA-X model of overgeneral memory. However, the evidence that supports these models is the result of disperse neuroimaging studies that have used different samples and diverse methodologies and analyses in order to validate their hypotheses. As a result, it is very difficult to draw strong conclusions about the brain areas that are in the centre of the cognitive processes that appear altered in depression. It would be useful to investigate in more detail and in a more consistent manner, using neuroimaging, some of the key brain areas that seem to be implicated in depression in order to elucidate their role in the illness and also perhaps, provide pharmacological research with novel targets for the development of new treatments.

5 Depression and Anhedonia

Anhedonia refers to an individual's inability to gain pleasure from normally pleasurable experiences. It is a core clinical feature of depression, schizophrenia, and other mental illnesses. The term anhedonia, by clinical definition, considers impairments in both reward motivation -motivational anhedonia- as well as a reduced ability to experience pleasure- consummatory anhedonia (Argyropoulos and Nutt, 2013). Several questionnaires as well as tasks have been developed in order to measure motivational and consummatory anhedonia which are both present in depression. Here, we will provide more details about the two components of anhedonia as well as the methods that have been used to capture these in depression.

5.1 Consummatory anhedonia

There are two main ways to capture consummatory anhedonia: a. via the administration of questionnaires that are asking volunteers to estimate how much pleasure they would experience in response to a given event and b. via different tasks that deliver a pleasurable stimulus and ask volunteers to rate how much they like it.

The SHAPS (Snaith-Hamilton Pleasure Scale) is a brief assessment scale for the estimation of the degree to which a person can experience pleasure or the anticipation of a pleasurable experience. The scale consists of 14 items that relate to common experiences which include interest/pastimes, social interaction, sensory experiences and food/drink which are most likely to be enjoyable to most people (Snaith et al., 1995). The SHAPS has been used to assess hedonic capacity among adults with major depression (Nakonezny et al., 2015) but also to examine the antidepressant effect of different pharmacological compounds in depressed patients (Lally et al., 2014, Gargoloff et al., 2016). Studies that have conducted a psychometric evaluation of this scale in large cohorts of patients with MDD have concluded that the scale has excellent internal consistency, with construct validity, and is unidimensional in assessing anhedonia among adults with MDD (Nakonezny et al., 2015).The scale can be found in Appendix E.

Questionnaires such as the SHAPS however, ask volunteers to imagine how much pleasure they would draw from a particular experience and rate it accordingly. When measures of "in the moment" experience of pleasure were compared between depressed volunteers and healthy controls no differences were detected between the two groups (Sherdell et al., 2012). In the case of measuring the "in the moment" experience of pleasure, the rating was taken right after a specific reward was delivered. This could imply that depressed individuals might have difficulties in imagining how much pleasure they would draw from a given experience when in fact they would enjoy certain activities as much as healthy volunteers (Sherdell et al., 2012). The low mood of these individuals as well as their bias towards negative thinking could also be contributing to that effect.

5.2 Motivational anhedonia

The most common way to assess motivational anhedonia is to examine how much effort volunteers are willing to make in order to obtain a specific reward. Both the EEfRT (Effort Expenditure for Reward Task) as well as the MID (Monetary Incentive Delay) task have been used to measure motivational anhedonia in healthy populations as well as mental health patients.

The EEfRT requires participants to decide how much effort they are willing to invest for different probabilities of obtaining a specific reward (Treadway et al., 2009). The MID task is a reaction time task which requires individuals to perform fast enough button presses as a response to a specified stimulus, in order to obtain a monetary reward. A specific cue that appears before the stimulus, indicates the magnitude of the reward they will receive if they make a sufficiently quick response when the stimulus appears (Knutson et al., 2000). The MID task has been used in neuroimaging studies to measure motivational anhedonia in patients with MDD as well as schizophrenia (Keren et al., 2018, Nielsen et al., 2012).

In the paragraphs that follow we will try to describe the neuronal components of reward processing and anhedonia in healthy volunteers, mainly drawing evidence from neuroimaging studies bit also animal research. Furthermore, we will try to explain how these processes could be altered in depression and linked to some of the symptoms of the disorder. Finally, emphasis will be given on the MID task and how it has been used to study the neuronal correlates of reward processing.

5.3 Neuronal components of anhedonia

Consummatory as well as motivational anhedonia are extremely complex constructs that encompass several neuronal processes. Impairments in reward anticipation as well as in the integration of information required in order to outweigh the costs and benefits of specific rewards along with low mood and bias towards negative thinking could all contribute to deficits in the individual's hedonic capacity (Rizvi et al., 2016). The neuronal pathways that underlie these processes are rather complex and could involve several neurotransmitter systems as well as an interplay between many brain areas. Most of our knowledge around the brain regions that are important for reward processing comes from animal studies(Moreau, 2002, Scheggi et al., 2018) but also more recently from neuroimaging studies (Enneking et al., 2019, Gorwood, 2008). Most animal behavioural experiments as well as human neuroimaging studies use tasks designed in a way which allows researchers to examine separately the anticipatory phase of getting a reward as well as the actual gain or loss of a specific reward, feedback phase.

5.4 Reward processing in the healthy brain

In order to understand anhedonia and how changes in the activation of different brain regions during depression could contribute to deficits in reward processing, we need to firstly understand reward processing in the healthy brain. Reward processing in the brain is an iterative learning process involving goal directed behaviour and adaptive decision making in response to a stimulus. Stimulus presentation, followed by receipt of reward, increases the likelihood of a behaviour occurring again (O'Doherty, 2017). The anticipation of a reward creates motivational salience, and the consumption of the reward reinforces motivational salience.

The neuronal substrates of reward processing in the brain as they have arisen form research in both humans and animals include the ventromedial prefrontal cortex - encompassing orbital and medial prefrontal regions- the amygdala, the striatum and the dopaminergic midbrain. These highly interconnected brain regions form a "reward network" (Wilson et al., 2018). Several regions of this network along with other brain areas are activated both during the anticipation of an expected reward as well as the receipt of an expected or unexpected reward.

5.5 Brain areas that are important for stimulus reward value, predictive reward value and prediction error

The orbitofrontal cortex and the amygdala are the two main brain areas that have emerged from the literature as activated during coding for stimulus reward value. Human neuroimaging studies show that the orbitofrontal cortex is activated while coding reward value for different types of sensory stimuli, including olfactory, somatosensory, auditory, and visual cues but also during the coding of reward value of more abstract stimuli such as monetary rewards (Rolls, 2000, Rushworth et al., 2011). The activity of this brain area has been shown to increase while attributing reward value to specific cues. However, after receipt of the actual reward activity in the orbitofrontal cortex decreases indicating a unique role of that brain area during the learning phase of the reward value of a stimulus (Noonan et al., 2011).

The amygdala is another brain area that is important during the processing of reward stimuli. This brain region is activated during the presentation of both pleasant as well as aversive stimuli (Morrison and Salzman, 2009). Experiments that have examined the activation of the amygdala during the presentation of rewarding stimuli that were matched for valence but differed in intensity and vice versa show that the amygdala seem to be particularly sensitive to the difference in the intensity of valence-matched rewards (Murray, 2007).

In addition to responding to a rewarding and/or punishing stimulus, it is also important to be able to predict in advance when a reward or punishment would occur so that our behaviour could be organized prospectively. Prediction of the value of specific reward or punishment involves activation of the orbitofrontal cortex, the amygdala as well as the ventral striatum (Murray and Izquierdo, 2007). These brain regions are activated during the presentation of different stimuli that have been associated with specific rewards and thus have predictive value for those rewards.

A very interesting research question relates to how the brain acquires predictive value representations. Research done in primates indicates that learning occurs through prediction error which signals the discrepancy between expected and actual reward or punishment. The phasic activity of dopamine neurons has emerged from primate research as possible neuronal substrate of that signal (Schultz, 2016b, Schultz et al., 1993, Ljungberg et al., 1992). Unexpected omission of a reward after a presentation of a stimulus that could have predictive value for that reward results in a decrease in the neuronal activity form

baseline (negative prediction error). The unexpected presentation of a reward however, after omission and/ or presentation of the stimulus with predictive value for that reward, results in an increase in the neuronal activity of dopaminergic neurons from baseline (positive prediction error). Over the course of learning, dopamine neurons that initially fire during the actual receipt of the reward or punishment shift their activity to the anticipatory phase of the reward which is signalled by the presentation of a stimulus with predictive value (Ljungberg et al., 1992, Schultz, 2016a, Schultz et al., 1993).

Functional MRI (Mori et al., 2019, Wolke et al., 2019) studies as well as PET (Dubol et al., 2018, Schott et al., 2008, Urban et al., 2012) studies have shown activation in prominent target areas of dopamine neurons as well as increases in the dopamine release in the striatum during reward prediction. However, striatal activity seems to be very important not only for prediction errors but also for coding the salience of a stimulus as well as during aversive learning. Moreover, striatal activity has also been detected during the presentation of non-rewarding stimuli such as distractor cues and the omission of a stimulus could also cause deactivation of some parts of the ventral striatum, especially when the omission of the stimulus is then followed by punishment (Schultz, 2016b, Schultz, 2016a). These findings indicate that striatal activity is not unique to reward prediction but is necessary for coding the saliency of different stimuli and could also mediate affective processing.

5.6 The MID task

The MID task was firstly developed during the latter half of the 1990s. During that time neuroimaging techniques were starting to emerge, and the MID was particularly designed in order to leverage the spatial and temporal resolution of fMRI to localize affective responses in the brain. The task examines reward processing, when the possibility of gaining money is offered to participants (Knutson et al., 2000). The use of money as a stimulus for this task is rather convenient. Money could be assigned either a positive value- money could be gained – or a negative value- money could be lost. Moreover, it could be cued as well as delivered and as such the MID task allows reward processing to be parsed into at least two distinct components namely, “anticipation” and “feedback”. Finally, monetary rewards could be assigned different attributes signifying valence, magnitude and probability and thus could allow for a more elaborate distinction of the different processes that take place in the brain during reward learning (Breiter et al., 2001).

Since its development,t the MID task has been extensively used in neuroimaging research and several meta-analyses have tried to summarize the research that has already been conducted using that task. According to the meta-analysis conducted by Wilson et al (2017) and which focuses only on the reward anticipation phase of the task, brain areas that are part of the salience network appear to be strongly activated or deactivated when anticipation of an actual reward is contrasted to the neutral condition of the task. These brain areas include the anterior insula and anterior and posterior cingulate cortex along with the NAc (Nucleus Accumbens), the caudate, the putamen as well as the cerebellar vermis. Specifically, the anterior insula presented with bilateral deactivation during reward anticipation. The insular cortex is considered a major cortical target of ascending interoceptive and visceromotor signals which pass through thalamic nuclei. The insular cortex is also functionally connected to the amygdala, the dorsomedial thalamus and the hypothalamus periaqueductal grey matter (Wilson et al., 2018).

The core of the DMN, the posterior cingulate cortex, was also bilaterally activated during reward anticipation (Andrews-Hanna, 2012). The PCC could be implicated in the monitoring of the environment and its activation could underlie learning and memory retrieval processes that are necessary in order to establish the links between the stimuli and the rewards that they are supposed to represent. Activation in that region could also signal a positive self-appraisal upon receipt of a reward (Andrews-Hanna et al., 2014, Yeo et al., 2011). Many temporal brain areas such as the parahippocampal gyri as well as the left hippocampus form part of the pattern of brain areas whose activation changes during reward anticipation. Activation in these regions could be associated with salience processing(Seeley et al., 2007). The cerebellum is known to have an important role for motivational salience, and animal studies have suggested a role in encoding expectation of reward (Cutando et al., 2013). The MID is also a motor processing task and as such broad bilateral activation of the primary motor cortex is observed, including the somatosensory cortex, the supplementary motor area and the multiple thalamic nuclei.

It is worth mentioning the lack of significant activations in brain regions such as the amygdala, which have emerged from the literature as important for reward anticipation during the MID (Hommer et al., 2003). This does not mean that these brain regions are not important for the task, but it might be that they do not present with consistent changes in their activation amongst all individuals performing the MID and thus do not survive the statistical cut off of the meta-analysis and research that examined the task in healthy voluneers.

Although most of the literature around the MID task focuses on the reward anticipation phase of the task, several studies have look at the actual reward outcome-feedback- of the task. The brain regions whose activation is altered during delivery of a reward compared to the neutral condition, include the OFC (Orbital Frontal Cortex) and the vmPFC (ventral medial Prefrontal Cortex) as well as the PCC (Oldham et al., 2018). The OFC is potentially engaged in processing the value of gain and the vmPFC in encoding and strengthening the relationship between the stimulus and its outcome (Kennerley and Walton, 2011). These two brain areas have previously been identified in the literature as important for the delivery phase of an abstract reward, like money (Breiter et al., 2001, Kennerley and Walton, 2011). The activation of these brain regions could be associated with the subjective experience of pleasure. Dysfunction in these regions has also been linked to anhedonia (Rolls, 2000).

6 Altered reward processing in depression

The MID task, as mentioned before, has been extensively used in neuroimaging studies that aim to investigate reward processing in healthy individuals. The task has also been used in order to understand how the neuronal activity underlying these process could be altered in specific disorders. Anhedonia, and consequently altered reward processing, is one of the most common and persistent symptoms of depression and several neuroimaging studies have used the MID along with other reward tasks to investigate how reward anticipation and feedback could be altered in depression. Here we will provide an overview of these studies.

One of the first studies to examine affective cognition in depression used PET (Positron Emission Tomography) to scan depressed patients and healthy controls performing the Tower of London task. The task has three feedback phases, one with positive, one with negative and one with no feedback at all. Responses in the medial caudate and ventromedial OFC to positive and negative feedback were blunted in depressed patients, supporting studies that have found blunted behavioural responses to reinforcement in MDD (Elliott et al., 1997).

Similar studies have also replicated these findings using different tasks. When the feedback phase of the MID task was examined by Pizzagalli et al. (2008) they found attenuated VS responses in depressed patients compared to healthy controls. These results were interpreted by the authors as evidence of the reduced hedonic capacity which primarily involves the NAc. This finding was also accompanied by a poorer overall performance in the task from depressed individuals (Pizzagalli et al., 2008). Reduced VS responses have also been identified during positive feedback of a gambling task in which depressed patients performed significantly worse (Must et al., 2013). Taken together, these findings could indicate that frontostriatal deficits could be associated with the failure of depressed patients to learn from feedback and adequately perform these tasks. Reduced VS responses were also identified in treatment naïve MDD patients during anticipation of gain and loss in the MID task and 6 weeks of treatment with escitalopram were able to reverse that hypoactivation suggesting that some of the reward related deficits could be reversed after treatment (Stoy et al., 2012).

A recent study using primary rewards, such as chocolate, suggested that frontostriatal dysfunction could be a trait marker for depression, persisting even during remission. Moreover, this altered brain activity observed in MDD could be denoting risk for the disease even in the absence of symptoms. Compared to healthy control subjects, remitted depressed patients showed decreased responses to primary rewarding stimuli in the VS, caudate, and cingulate cortex, even though their subjective ratings and taste cortex responses were the same. In response to primary aversive stimuli (e.g., moldy strawberries), however, the between-group differences were more complex: enhanced responses in the caudate and blunted responses in the lateral OFC (McCabe et al., 2010). Such pattern of increased subcortical responses and flattened PFC responses might explain how patients can have both an automatic negative bias but also an inability to integrate this information into appropriate behavioural plans. These findings are particularly compelling because they suggest that remitted patients, who lack clinical symptoms, nonetheless exhibit reinforcement–processing abnormalities, potentially contributing to their elevated risk for future depressive episodes.

Besides to responding differently to reward and punishment, depressed patients also show altered neural responses during anticipation of reinforcement. When unmedicated depressed patients and control subjects were scanned during the MID, it was shown that during reward anticipation the activation in the dorsal anterior cingulate cortex (ACC) increased in patients with MDD when they were anticipating increasing gains. Control subjects however, showed increased dorsal ACC activity when anticipating increased loss (Knutson et al., 2008). The authors argued that, given the role of the ACC in uncertainty or conflict, healthy individuals experienced affective conflict during anticipation of avoidable losses, whereas depressive subjects experienced affective conflict during anticipation of attainable gains (Botvinick et al., 1999, Carter et al., 1998). Alternatively, the dorsal ACC response might simply be a marker of dysfunctional reward-related decision-making on the part of depressed patients (Drevets et al., 2008b), although patients did not differ from control subjects behaviourally. Importantly, a subsequent study using a gambling task did not replicate the dorsal ACC finding but did find reduced striatal responses in MDD patients while anticipating reward.

One way to interpret these neuroimaging findings is in terms of dysfunctional network integration, particularly top-down control. For example, in a reversal learning task where participants had to ignore occasional misleading negative feedback, Taylor Tavares *et al.,* found that, compared with control subjects, depressed patients showed reduced PFC responses during response reversal as well as greater amygdala responses after misleading negative feedback. Furthermore, in control subjects but not in depressed participants, PFC and amygdala responses correlated with the ability to withhold an incorrect switch after misleading feedback(Taylor Tavares et al., 2008). Thus, maladaptive responses of depressed patients in this task might be related to impaired PFC-amygdala functional integration during negative feedback. This explanation complements a finding from Siegle *et al.*, who reported elevated amygdala responses when rating emotional words and reduced dorsolateral PFC responses when sorting digits in depressed patients (Siegle et al., 2007). These findings indicate that further research is required in order to better explore whether depression is associated with abnormal functional integration between PFC and limbic region.

Antidepressant action of ketamine

7 General overview of the most common treatments of depression

Antidepressant medication and mainly SSRIs (Serotonin Reuptake Inhibitors) and MOIS (Monoamine Oxidase Inhibitors) are the mainstream treatment for MDD. However, there remains considerable debate about the effectiveness of those groups of antidepressant treatments and the potential differences on effectiveness and tolerability between groups of antidepressant medication. This is mainly because the short-term benefits of antidepressants are on average, modest; the antidepressant effects take weeks or months to demonstrate and the long-term balance of benefits and side-effects is often understudied.

A recent meta-analysis investigated the efficacy and acceptability of 21 commonly prescribed antidepressant treatments in adults with MDD. The compounds included in the meta-analysis have different mechanisms of action that target different receptor systems and were all compared to placebo (Cipriani et al., 2018).The meta-analysis showed that antidepressant treatment was in general more efficacious than placebo. However, detectable antidepressant effects of those drugs were usually reported after several weeks of treatment. The considerable delay between initiation of antidepressant treatment and the improvement of depressive symptoms could, at least partly account, for the relatively high drop-out rates that were reported in most treatment trials. Participants who drop out of trials early tend to have poorer responses than those who remain on treatment till the end of the trial and this could obscure the findings on the effectiveness of these treatments.

The novelty of the treatment is another an important factor for the efficacy of the compound since it was shown that antidepressant medication tended to have better efficacy profiles when they were novel and mostly when they were used as experimental treatments, compared to when they had become old (Cipriani et al., 2018). The results of this meta-analysis along with other research investigating antidepressant treatments further emphasize our poor understanding of the factors that could contribute to the efficacy and acceptability of treatments and could perhaps lead to the development of novel antidepressant medications.

Research for novel and more effective antidepressants, has traditionally targeted the monoamine neurotransmission systems. Blockade of noradrenaline, dopamine and /or serotonin reuptake by acting on selective transporters (NET, DAT and SERT respectively) and inhibition of monoamine break-down by monoamine oxidase enzyme are the most established mechanisms of pharmacological action for antidepressant drugs (Feighner, 1999, Ferguson, 2001). However, other drugs that reduce NMDA receptor function and thus modulate glutamatergic transmission, present with antidepressant-like effects (Mathews et al., 2012).

Relatively recently, ketamine, a glutamatergic receptor antagonist has emerged as a potent, fast acting antidepressant. The US FDA has granted ketamine "breakthrough therapy" designation and has in March 2019 approved the nasal spray (esketamine), in conjunction with an oral antidepressant, for treatment of depression in adults that have tried other antidepressant medications but have not benefitted from them. In order to understand, however, how ketamine and other glutamatergic agents could exert their antidepressant action, we need to give a brief overview of the glutamatergic system focusing on the Central Nervous System.

8 Glutamatergic system- Alternative treatments for depression

8.1 The glutamatergic system

Glutamate is the main excitatory neurotransmitter in the mammalian CNS. Glutamate mediates its effects via two classes of receptors, the fast-acting ligand gated ion channels (ionotropic receptors) and the much slower in response G-protein coupled receptors (metabotropic receptors). The binding of glutamate on both classes of receptors is responsible for the immediate basal excitatory neurotransmission however, activation of the glutamate receptors has also been implicated in longer-lasting neuronal processes such as LTP (Long-Term Potentiation) and LTD (Long-Term Depression). LTP and LTD are very important neuronal processes with a central role in learning and memory thus, compounds that target the glutamatergic system could have potential therapeutic effects in mood disorders, cognitive impairment (ex. Alzheimer's disease) and condition such as epilepsy that could arise from excess excitation(Lamsa and Lau, 2019).

The glutamate gated ion channel receptors could be subdivided in to three main families of receptors: AMPA receptors, kainate receptors and NMDA receptors (Gunduz-Bruce, 2009). Ketamine acts as an NMDA receptor antagonist and as a result focus will be given on the NMDA receptor subgroup (Mathews et al., 2012). By converging specific patterns of neuronal activity into long term changes in synaptic structure and function, NMDARs play a very important role in mnemonic processes and brain plasticity. At a molecular level, NMDARs assemble as heterotetramers consisting of two GluN1 and two GluN2 subunits (Gunduz-Bruce, 2009).

The GluN1 subunit is expressed in the CNS at all stages of development whereas the GluN2 subunit has four different subtypes: GluN2A, GluN2B, GluN2C and GluN2D. The GluN2 subunit subtypes have differential developmental and anatomical profiles (Zhu and Paoletti, 2015). The diversity in the expression, and consequently the function, of the GluN2 subunit subtypes confers NMDARs with distinct biophysical and pharmacological properties. In the embryonic brain the GluN2B and GluN2D subunits predominate while the GluN2A and GluN2C are absent and their expression starts right after birth (Paoletti et al., 2013). Anatomically, the GluN2A and GluN2B subunit subtypes are mainly expressed in the forebrain while the GluN2C subunit is mostly confined in the cerebellum and the GluN2D subunit is mainly restricted in areas of the midbrain, specifically, the hippocampus, amygdala, ventral nuclei of the thalamus and the olfactory bulb (Paoletti et al., 2013, Zhu and Paoletti, 2015).

At a subcellular level, diverse NMDAR subtypes co-exist at the level of different neuronal types, but their expression also varies within individual neurons and even at individual synapses. In most neurons the density of the NMDARs is higher in dendritic spines, within the post-synaptic shaft and the somatic membrane. In the perisynaptic and extrasynaptic spaces NMDA receptors that are enriched with the GluN2B subunits predominate (Gunduz-Bruce, 2009).

8.2 The role of the glutamate system in depression

Drugs that target the glutamate receptors have been speculated to have antidepressant action before there was evidence that the glutamatergic system might be important for mood disorders (Mony et al., 2009). Animal models of depression as well as the neuroimaging techniques in humans along with the use of drugs that could modulate glutamatergic transmission have more recently allowed us to shed some light on the rather complicated role of the glutamatergic system in depression. Here we will provide an overview of the role of the glutamatergic system in depression, drawing evidence from animal research and neuroimaging human studies. We will also give a brief overview of the glutamatergic drugs that have been tested as potential antidepressant treatment. Finally, we will focus on ketamine, its possible mechanism of action in the molecular, cellular and neuronal level, as well as the potential models that could explain its unique antidepressant profile.

8.3 Altered brain anatomy and function in depression: evidence from preclinical models and human neuroimaging studies

Chronic stress animal models have been used in research in order to examine key features of depression. Preclinical studies in rodents and non-human primates have reported structural brain changes in areas including the hippocampus and PFC and these alterations were mainly present in chronic stress models (Krishnan and Nestler, 2011, Lucassen et al., 2014). Decreased grey matter volume has also been reported in the hippocampus and PFC of depressed patients using neuroimaging (Drevets et al., 2008a, Grieve et al., 2013). These brain areas receive dense glutamatergic input and the loss in grey matter volume correlated with the severity of the illness and the duration of treatment. There is also evidence that this decrease could be reversed after successful treatment (Drevets et al., 2008a). In animal models of chronic stress, there is also evidence that the loss of hippocampal and PFC volume could be reversed after discontinuation of the stress factors (Grieve et al., 2013). Structural changes, mainly volume decreases have also been observed in the insula whereas depression has been associated with increased amygdala volume (Drevets et al., 2008a).

Functional brain imaging studies have also reported several alterations in the connectivity patterns of the main brain networks of depressed patients compared to heathy controls. The majority of these studies indicate that the DMN presents with increased connectivity in depressed individuals compared to healthy controls whereas the SAL (Salience Network) and CEN (Central Executive Network) networks present with decreased connectivity (Helm et al., 2018). These findings are consistent with increased rumination and introspection that characterises most of depressed individuals as well as a decreased association with external stimuli (Hamilton et al., 2015). A potential issue however, that could arise from neuroimaging studies that recruit depressed patients is the understudied effects that antidepressant medication could have on the connectivity of brain networks during rest. As a result, in research studies where depressed patients were scanned while on antidepressants, it is difficult to disentangle which of the differences in brain connectivity are due to the nature of depression and which could be a potential effect of antidepressant treatment (McCabe and Mishor, 2011, McCabe et al., 2010).

In an effort to understand the connectivity changes that occur in depression without having to take into consideration the effect of treatment we summarised all the connectivity studies that have included depressed patients who were drug-naïve at the time of the scan or have been abstaining from antidepressant medication for at least 3 months prior to scanning (Table Summary in Appendix A). The DMN has been a central focus point for most of these studies however, there are no consistent changes that could be identified either between as well as within the DMN regions (Zhu et al., 2012, Zhu et al., 2017) Moreover, studies that have used different connectivity methods failed to replicate this finding. For example, a study that have used an ICA approach did not significant connectivity changes in the DMN between depressed individuals and healthy controls (Veer et al., 2010). The findings from most of these studies are not easy to summarise. Both increases as well as decreases in the connectivity between several brain networks as well as brain regions within the same network have been identified. Moreover, the different experimental hypotheses that each study has chosen to investigate along with the different methodologies that have been implemented makes it even harder to draw conclusions.

Changes in the connectivity in MDD could be related to disruption in the glutamate/excitatory neurons of these brain areas which could also contribute to the structural alterations that are observed in these areas (Helm et al., 2018). In addition, these brain regions also receive extensive GABAergic input. The interplay between glutamatergic excitatory neurons and inhibitory GABAergic interneurons which determines the balance between inhibition and excitation in frontal brain areas is considered of great importance during depression (Tremblay et al., 2016). In models of chronic stress, the strongest evidence for altered glutamatergic transmission comes from morphological changes in excitatory principle neurons. This change in glutamatergic transmission demonstrates as a decrease in the dendritic length and branching of hippocampal pyramidal neurons (Duman et al., 2019). Chronic stress also decreases the number and function of synapses in pyramidal cells. These findings could indicate that chronic stress could decrease the structure and number of glutamatergic neurons (Duman et al., 2019).

Preclinical studies of chronic stress also report reductions in the levels of GABA synthetic enzymes and neuropeptides in medial PFC and other cortical brain regions. The alterations in GABA levels could influence the function of the three major and non-overlapping GABA interneuron subtypes (Banasr et al., 2017, Luscher and Fuchs, 2015). These interneurons are classified based on their morphology, as well as electrophysiological and molecular characteristics, particularly the expression of SST (somatostatin), PV (parvalbumin) or 5-HT3a receptors. Chronic stress models the SST and PV neurons are mostly affected. SST interneurons target dendrites and regulate their excitability. PV interneurons target the soma and initial axons of pyramidal neurons(Tremblay et al., 2016). Vulnerability of the GABA interneurons could influence the tonic activity of these neurons and affect the excitatory output that these interneurons control. These alterations in the balance between excitation and inhibition could be accountable, at least partly, for the connectivity changes observed in depression and makes these two neurotransmitter systems potential targets for the treatment of depression (Thompson et al., 2015).

8.4 Glutamatergic agents as antidepressants

Several compounds that target the glutamate system have been tested for their antidepressant actions. D-cycloserine, a tuberculosis antibiotic, was one of the first compounds with action on the NMDA receptors that was shown to produce significant mood improvement in depressed patients. In 1959 Dr George Crane reported the effects of D-cycloserine in mood improvement of depressed patients within two weeks of treatment. More than a decade after Crane's findings, a placebo-controlled trial confirmed the initial findings by reporting a significant progressive improvement in the depressive symptoms of patients over 6 weeks of treatment with D-cycloserine (Heresco-Levy et al., 2013). This compound has a very unique mechanism of action since it acts as a partial agonist on the NMDARs that contain GluN2A and Glun2B subunits and as a full agonist on NMDARs that consist of the GluN2C and GluN2D subunits (Sheinin et al., 2001).

The next agent that was studied was amantadine, a partial NMDAR antagonist which is used for the treatment of Parkinson's disease. Another similar compound is memantine which was also tested for its antidepressant properties. However, the outcomes of these studies did not yield very promising results and were rather inconclusive (Vale et al., 1971). Another low affinity NMDAR antagonist that shows promising antidepressant effects is AZD6765. The human trials for this compound produced promising results (Zarate et al., 2013). Following these early clinical studies, animal models of depression were developed and used to access the antidepressant effects of several glutamatergic compounds (Pilc et al., 2013).

9 Ketamine

In the last decade, the non-competitive NMDAR antagonist ketamine, has emerged as a fast-acting and robust antidepressant by improving core depressive symptoms, including anhedonia, which is not usually targeted by conventional treatments (Salvadore and Singh, 2013). Remarkably, these actions are observed within hours after a single ketamine dose IS administered and persiste on average for 1 week. Since the results of the first ketamine study were published by Berman et al 2000 several other studies have been conducted in order to assess the tolerability of single as well as repeated ketamine administration in different patient populations (Berman et al., 2000, Zarate et al., 2006, Strong and Kabbaj, 2018). Moreover, possible ways have been investigated to maintain the antidepressant response of the drug (Chiu et al., 2014, Strong and Kabbaj, 2018). In order to better evaluate the findings of these studies we need to understand the pharmacology of ketamine and what is known so far about its mechanism of action.

9.1 Pharmacology of ketamine

Ketamine was first developed in the 1960s and is still used as an anaesthetic. Ketamine is a racemic mixture of R- and S-enantiomers. The S-enantiomer, also known as esketamine, displays an in vitro approximately fourfold greater affinity for the NMDA receptor compared to R-ketamine (Zanos et al., 2016). In human studies, esketamine has a greater analgesic and anaesthetic activity with less psychotomimetic effects than the racemic mixture or the R-enantiomer and therefor has a greater potential efficacy and tolerability as an antidepressant (Morris et al., 2017, Zanos et al., 2016). The nasal spray that has currently been approved as an MDD treatment by the FDA concerns esketamine. In animal models of depression however, R-ketamine shows higher and longer – lasting antidepressant potency when compared to S-ketamine (Zhang et al., 2014). The mechanism of action of ketamine is not currently entirely known. Most recently, it was proposed that the antidepressant activity of ketamine could be related to the R-enantiomer and more specifically to one of the metabolites of R-ketamine and specifically (2R,6R)-HNK (Hydroxynorketamine) (Zanos et al., 2017).

Ketamine could be administered intravenously, intranasally, intramascularly, sublingually, rectally, orally and topically. In the liver, ketamine undergoes extensive 1st pass metabolism by liver enzymes to norketamine via N-methylation. Other ketamine metabolites include hydroxyketamine, dehydroxyketamine and hydroxynorketamine (HNK). Ketamine's plasma half-life is between 1h and 3h and norketamine's plasma half-life is 12h. After ketamine administration (2S, 6S-2R, 6R)-HNK is the major metabolite found in the plasma and brain of mice as well as humans (Clements et al., 1982). In the section that follows we will try to describe the mechanism through which ketamine exerts its antidepressant action at the molecular and neuronal level.

9.2 Neuronal and molecular mechanism

Upon administration, ketamine's main metabolite noreketamine, binds on and causes continued blockade of NMDARs. The blockade of NMDARs by ketamine results in increased glutamate efflux, which would then stimulate non-NMDA glutamate receptors such as AMPA and kainate receptors (Gould et al., 2019). The functional antagonism of the NMDARs expressed in GABAergic neurons has been postulated as the main mechanism through which ketamine causes its dissociative, psychosis-like effects whereas the activation of AMPA receptors is particularly important for the antidepressant action of the drug (Zanos et al., 2018). The NMDA blockade disrupts glutamatergic inhibition not only in GABAergic but also serotonergic and noradrenergic neurons thus leading to disinhibition of major excitatory pathways and increased extracellular glutamate levels (Zanos and Gould, 2018) .

Although ketamine has the same and relatively low affinity for all the different subtypes of NMDA receptors, NMDA receptors that contain the GluN2B subunit are considered to be particularly important for the antidepressant actions of the drug (Gould et al., 2019). Compounds that selectively act on this subunit, for example Ro25-6982 and CP101.606 also demonstrate antidepressant action (Nash et al., 2004, Poleszak et al., 2016). The GluN2B subunit is mostly expressed at the extrasynaptic sites and seems to mediate the phosphorylation of eEF2 (eukaryotic elongation factor 2), BDNF (Brain Derived Neurotrofic Factor) as well as mTOR (mammalian Target of Rapamycin). Phosphorylation of all these factors activates molecular cascades that lead to an increase in synaptic density as well neurogenesis (Scheuing et al., 2015).For the potential mechanisms of ketamine's antidepressant action see Figure 1.

Depression, as mentioned before, is characterised by atrophy of pyramidal neurons as well as loss of the dendritic spines leading to reduced synaptic activity (Duman and Duman, 2015). The glutamate increase that accompanies ketamine's administration is followed by an increase in the number of spine synapsis and rapid reversal of the effects of chronic stress in animal models of depression (Scheuing et al., 2015). The activation by ketamine, of molecular pathways that could reverse those deficits could explain the antidepressant action of ketamine at the cellular and molecular level. Moreover, synaptogenesis requires the transcription and translation of new proteins which could at least partly explain the delay in the initiation of ketamine's antidepressant action but also its relatively long-lived antidepressant effects (Zanos and Gould, 2018).

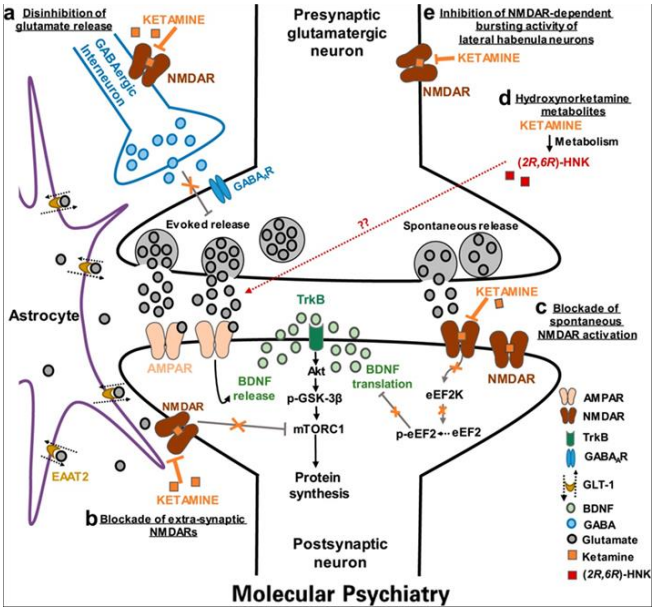

Figure 1 *Provides a summary of the proposed mechanisms of ketamine's antidepressant action (a, b, c, d and e). The two most prominent mechanisms propose that ketamine either via indirect inhibition through GABAergic interneurons (a) or directly via binding of (2R,6R)-HNK on AMPARs (d) leads to enhanced glutamatergic firing. Activation of the downstream molecular pathways could increase BDNF synthesis which is associated with sustained synaptic plasticity. This is necessary for the strengthening of the excitatory synapse and a sustained antidepressant response. The picture was published in (Zanos et al., 2018)*

The activation of the BDNF and mTOR pathways which promote protein synthesis and increase synaptic plasticity are thought to be mediated by ketamine via its indirect activation of the AMPA receptors (Henley and Wilkinson, 2016). Specifically, ketamine's binding on the inhibitory GABA-ergic interneurons could lead to the disinhibition of pyramidal neurons which induces enhanced glutamatergic firing. The excess glutamate would then bind on the AMPA receptors and thus initiate the activation of the BDNF and mTOR pathways (Duman et al., 2016). Recently however, a more direct mechanism for the

activation of AMPA receptors by ketamine has been proposed (Kavalali and Monteggia, 2018, Zanos et al., 2018).

This alternative mechanism for ketamine's antidepressant action involves one of ketamine's active metabolites, (2R,6R)-HNK. This metabolite which is the major metabolite found in the plasma and brain of mice as well as humans after ketamine administration, does not functionally inhibit the NMDA receptors but in hippocampal slice records, (2R,6R)-HNK causes a robust increase in AMPAR mediated excitatory post synaptic currents (Zanos et al., 2017). When HNK was isolated, a single dose of the metabolite was sufficient to produce a robust antidepressant effect in animal models of depression. Although some studies that have isolated (2R,6R)-HNK have failed to reproduce the aforementioned findings (Shirayama and Hashimoto, 2018), the idea that ketamine's antidepressant action could be linked with one of the more long-lived metabolites of the drug would explain the unique antidepressant profile of the compound and requires further thorough investigation.

9.3 Ketamine's effects in the brain: an insight from neuroimaging studies

In order to be able to understand the effects that ketamine produces in the brain we need to bear in mind that ketamine, depending on the dose of administration, could produce rather diverse and complicated pharmacological effects. In high doses ketamine is an anaesthetic that has been extensively used in paediatrics and veterinary medicine. In low subanaesthetic doses, the antidepressant properties of the drug initiate but with a 2h delay between the administration of ketamine and the initial detection of any mood improvement. Finally, in higher subanaesthetic doses the drug produces analgesia and psychotomimesis. The psychotomimetic effects of ketamine include delirium, dissociations and hallucinations. These effects appear shortly after the administration of the drug, peak around 15min after the start of an intravenous infusion and dissolve almost immediately after the end of the infusion (White et al., 1982).

The dissociative effects of ketamine which are present as part of the anaesthetic use, and also present when ketamine is administered at sub-anaesthetic doses, make the drug an excellent model for the positive symptoms of psychosis which have mostly been associated with underlying glutamatergic deficits (Moghaddam and Javitt, 2012). Several neuroimaging studies have looked at the acute/psychotomimetic effects of the drug by administering ketamine to healthy volunteers while they were lying in the scanner. Overall,

ketamine produced an increase in brain connectivity as demonstrated by GBC (Global Brain Connectivity) studies. When the connectivity of specific brain networks was examined, ketamine increased connectivity within the DMN, the visual and sensorimotor networks. The increased connectivity of these networks could account for the dissociative effects of acute ketamine administration as well as the visual and auditory hallucinations that sometimes occur (for review of these finding see (Frohlich and Van Horn, 2014)).

Research studies have demonstrated that subanaesthetic doses of ketamine could disrupt the encoding of semantic memories as indicated by the impaired retrieval of these memories(Honey et al., 2005). However, already encoded memories remain untouched by ketamine. The hippocampus plays a key role in memory formation and as such there is evidence that its activity would be modulated by ketamine (Honey et al., 2005). Several pharmacological neuroimaging studies have explored the effects of acute ketamine administration in hippocampal connectivity. These studies show that under ketamine, connectivity between the hippocampus and areas of the limbic system, such as the amygdala, but also brain regions including the precuneus, present with increased connectivity. Moreover, increases were identified between the hippocampus and the auditory and somatosensory networks (Mueller et al., 2018). These findings indicate that ketamine could alter hippocampal connections with brain areas that are important for emotional regulation and sensory perception. These processes have previously been discussed for their important role during the encoding and consequent retrieval of AMs.

9.3.1 The delayed effects of ketamine in healthy volunteers

Only two studies have examined the delayed effects of ketamine on brain connectivity of healthy volunteers (Lehmann et al., 2016, Scheidegger et al., 2012). Both studies have scanned individuals, 24h post the drug administration and special emphasis was given on the effects of ketamine on the connectivity of DMN. The ketamine doses that were used for these studies were sufficient to induce the antidepressant effects of the drug. In the study by Scheidegger et al (2012) a marked reduction in resting state functional connectivity between key nodes of the DMN was identified. These nodes included the PCC, the dorsal nexus, the pgACC as well as the MPFC. The decrease in the connectivity of the dorsal nexus, is particularly important since that brain area presents with increased connectivity with emotional processing and attention related networks (Sheline et al., 2010). Ketamine's effects on the dorsal nexus, in healthy controls, could indicate a potential antidepressant mechanism for ketamine (Scheidegger et al., 2012).

The second study that looked at the effects of ketamine, 24h post the drug administration, focused on the effects of the drug on areas that are key for resting state connectivity networks and are activated in emotional processing. An emotional processing task that involved positive and negative stimuli was presented to individuals and it was shown that increased activations in the pACC were present only during the processing of negative stimuli. This finding was more prominent in those individuals who presented with increased self-focus. Moreover, during the resting state, connectivity between the PCC and pACC was decreased. Since activation of the pACC, is important for emotional regulations (Northoff, 2005) and the decreased connectivity between the PCC and pACC could mediate a switch in individuals' attention from the self to external cues (Sheline et al., 2010), it was hypothesized that ketamine as an antidepressant, would attenuate a potentially pathological increase in self-focus (Lehmann et al., 2016).

9.3.2 The delayed effects of ketamine in depression

The results from ketamine's delayed effects- 24h post administration- on healthy volunteers, show that ketamine as an antidepressant could cause changes in functional connectivity, that would work towards reversing some of the changes in brain activity that are observed in depression. However, in order to be able to understand ketamine's actions as an antidepressant we need to examine the drug effects in depressed individuals. The literature examining the antidepressant effects of ketamine is in its infancy. Therefore we identified only two studies that used fMRI and examined the effects of ketamine 24h post the drug administration.

The first of these studies has used resting state to look at global brain connectivity in patients with MDD and healthy controls and examined the effects of ketamine on global brain connectivity 24h past the drug administration. The study identified reduced global brain connectivity in the PFC of patients with MDD compared to healthy controls at baseline, but increased connectivity in the PCC, precuneus and lingual gyrus as well as the cerebellum. Ketamine, 24h after its administration, significantly increased connectivity in the right lateral PFC and reduced connectivity in the cerebellum. Moreover, when the MDD group was split into ketamine responders and non-responders, it was shown that ketamine responders had increased connectivity in the lateral PFC, caudate and insula compared to non-responders (Abdallah et al., 2017).

In another study, ketamine's antidepressant effects, 24h after the drug administration were examined in a TRD (Treatment Resistant Group) group. The study used two separate emotion perception tasks to examine the effects of ketamine in patients with TRD and compare them to baseline. It was shown that ketamine increased neural responses in the right caudate and these increases were specific to positive emotion. Increased connectivity in the right caudate at baseline, was also associated with greater improvement in depressive symptoms after ketamine (Murrough et al., 2015).

Aims and hypothesis of our study

The findings from the neuroimaging studies that examine the effects of ketamine, 24h after the drug administration, although limited, seem to be mainly localised in areas that present with altered activations in depression and have a particularly important role in emotional processing and motivation for rewards (Abdallah et al., 2017, (Murrough et al., 2015). Some of these brain areas include the amygdala and the pACC as well as the caudate. This observation is in line with the cognitive models of depression, where altered emotional perception and focus on negative affect in combination to the increased self-focus of individuals, are the key factors that could contribute to the initiation and prolongation of a depressive episode (). However, in order to be able to understand how ketamine's antidepressant effects are mirrored in the brain and how this could relate to the improvement in depressive symptoms that ketamine produces, a better understanding of the altered neural processing in depression is required along with more research around ketamine's antidepressant action.

Although there are several fMRI studies in depressed individuals that focus on better understanding the brain activations that underlie altered cognitive and emotional processing in depression, these studies are often confounded by antidepressant treatment(). A possible solution to that problem would be the study of remitted depressed volunteers who might still present with some of the cognitive and emotional deficits of depression, such as rumination and anhedonia, but are asymptomatic and most often drug naïve.

As far as ketamine's antidepressant action is concerned, research has focused mainly on examining the changes in brain activity induced by the drug, at the peak (24 h post administration) of its antidepressant action (). Very little is known as to how the antidepressant effects of ketamine are mirrored in the brain when they are first detectable, 2h-4h after the infusion. It is also unknown whether these effects present differently in the brain, depending on the time point when they are examined or they remain unaltered through the window of ketamine's antidepressant action.

Our study is looking at medication free remitted depressed volunteers who receive ketamine (0.5mg/kg) and placebo (saline) intravenously, following the standard protocol for antidepressant treatment with ketamine. The aim of the study is to identify brain areas with known function in AM recall and anhedonia that might present with significantly altered brain activity 2h post the ketamine administration, compared to placebo. These brain regions could serve as neuroimaging markers for the early antidepressant effects of ketamine and could help us understand how the drug might affect rumination and anhedonia related processes in the brain.

Specifically, we have used the MID task to investigate how ketamine, 2h after its administration, would alter brain activity in areas that are associated with reward processing. Specifically, we have looked at the striatum, the amygdala, the insula and VTA. We hypothesise that ketamine, compared to placebo, would increase the activity of these reward processing areas. Moreover, we believe that this increase would be associated with the magnitude of the expected reward during the task. The VAMP task was specifically developed to examine rumination and recall of AMs with different emotional valence (positive, negative, neutral) in our study. We believe that ketamine, would decrease the activation of the brain areas such as the PCC, the sgACC and the amygdala, that are associated with emotional processing and have a known role for AM recall.

Methods

Participants

The current study was conducted in remitted, depressed individuals. 36 volunteers aged between 19 and 47 years, with a confirmed history of depression completed the study. All participants were in remission at the time of the scan and free from any antidepressant medication. A minimum of three months of no antidepressant treatment was required before participants could be included in the study (the CONSORT in page 84 provides the exact number of screened and randomised participants). The sample size for this study was determined after conducting a power analysis that would allow us to investigate the effects of ketamine, compared to placebo, in the whole sample but also examine the effect of session by identifying any potential differences in the behaviour and brain activations of those participants who had ketamine on their first session, compared to placebo. Our power analysis was based on the activations produced by a very similar autobiographical memory task in the amygdala and the hippocampus (Nicholson et al., 2016).

In more detail, our inclusion criteria required at least one confirmed episode of depression and a current state of remission. The exclusion criteria involved: any history of other psychiatric or neurological disorder, except depression; a previous adverse response to ketamine; any medical conditions that affect hepatic, rhenal or gastrointestinal functions as assessed using standard laboratory-based blood tests and urinalysis; cardiac abnormalities as identified by a standard 12-lead ECG; hypertension; a significant history of drugs of abuse or positive drugs of abuse test; excessive use of nicotine (>= 5 cigarettes/day), alcohol (>=28 units/week) and caffeine (>=6 cups/day); other MRI contraindications.

Our study participants were identified via advertisements mainly posted at the King's College internal website dedicated to research (https://www.kcl.ac.uk/ioppn/depts/pm/research), but also newspaper advertisements and word of mouth. Those who expressed an interest in taking part in the study were approached mainly via e-mail and were provided with a copy of the information sheet. A telephone screening followed, to establish the suitability of those interested to continue with the study. The volunteers that successfully passed the telephone screening were then invited for a screening visit at the CNS (Centre for Neuroimaging Sciences). More details about the screening and study days are provided in the section that follows.

Study design

The current study adopted a randomised, double-blind, placebo controlled, within subjects, cross over design for the investigation of the delayed effects of ketamine in the brain. Volunteers suitable for this study needed to have suffered from depression in the past and were on remission at the time of the scan. The mental health history of the volunteers was assessed using the MINI (Mental International Neuropsychological Interview) version 6.0.0 (January1, 2006). The MINI was administered once, during the screening visit as indicated in the more detailed description of the study procedure that follows. The study was approved by King's College London's Psychiatry, Nursing and Midwifery Research Ethics Committee (REC Reference Number: HR-14/15-0650) and funded by Johnson and Johnson via a grant attributed to UCL. A copy of the ethics approval letter can be found in Appendix H.

Having responded to study advertisements, potential participants were asked a number of screening questions via telephone in order to establish their suitability for the study. They were then invited to the Centre for Neuroimaging Sciences (CNS) for a more detailed screening which lasted approximately 90min. The screening visit involved taking a detailed medical and mental health history (MINI), a urine test, a pregnancy test (when applicable) and a blood screen. Participants were also introduced to the tasks they would undertake before and during the MRI scan and were given the opportunity to practice them. On the screening day, volunteers were also introduced to the MR environment in the mock scanner and were asked to complete the RRS (Ruminative Response Scale), a rumination scale which measures how much participants ruminate about their positive and negative past experiences, especially during times when they feel sad or depressed (Treynor et al., 2003).

For those volunteers deemed eligible to participate in the study, arrangements were made for an interview which took place on a separate day and lasted approximately 1hour. During the interview participants were asked to describe, in a detailed manner, their most positive, their most negative and one neutral personal experience. The interviewees were encouraged to share experiences that dated up to 12 months prior to taking part in the study in order to ensure a detailed and accurate recollection of the positive and negative events. However, if something more positive and/or negative had taken place further back in time, participants could talk about that event provided that they could share a lot of details around it.

During the interview, participants were encouraged to talk freely about the events they were comfortable to share with the interviewers. However, when guidance from the interviewer was necessary, the questions used for that purpose were based on the LEDS (Life Events and Difficulties Schedule) interview which was especially adapted to the demands of this study. A copy of the amended LEDS version, used in our study, can be found in Appendix B. The interviews were recorded with the participants' consent and the events were rated independently by the study's medical doctor and the researcher, using the LEDS rating scale, for their objective emotional valence. Only "Severe" events were included in the study to ensure equal emotional valence for all events across all participants. During the study days, as part of a valenced-AM, personalized task (VAMP task), the statements from the most positive, negative and neutral event were presented to the participants in the scanner, and they were asked to think about those events and rate their emotional valence using a VAS (Visual Analog Scale) scale. More details about the LEDS interview, the LEDS rating scale and the VAMP task are provided in the sections that follow.

Two separate visits at the CNS followed the interview day and each of them was about 6 hours long. These two visits were arranged to the participants' convenience and were 7-14 days apart. The schedule was kept identical for both study days with only the drug administered on each day being different. Volunteers were given either ketamine or placebo. Initially, on each study day, participants underwent a medical examination and completed questionnaires that provided us with detailed information about their current mental and emotional state (see Table 1. for details on out-of-the-scanner assessments performed on each day). The RAVLT (Rey Auditory Verbal Learning Test) as well as part of the Wechsler Memory battery were administered in order to measure of the cognitive state of participants that we could compare between study sessions. A blood sample was also collected and was used to measure cytokine blood levels prior to ketamine administration (this analysis is not reported as part of this thesis). The participants were then administered ketamine (0.5mg/kg) or saline (placebo drug) intravenously with the help of a cannula.

The drug or saline administration lasted 45 min and was delivered via a programmable intravenous infusion pump following a continuous infusion protocol identical to that currently used for treatment of depression. Typical infusions, where ketamine is administered as an antidepressant, last for 40min. In our study however, we allowed an additional 5min in order to make sure that the blood sample used to measure the levels of ketamine and its metabolites was collected and enough time was given to participants in order to complete the tasks that were performed during the infusion. Twenty minutes after the infusion started- participants were asked to complete a neuropsychological test battery which comprised of two computerized tasks. The first task administered was the auditory hallucination task, which is designed to capture the ketamine experience. After this task, participants were asked to complete the EEfRT which measures their hedonic capacity(Treadway et al., 2009). At the end of the infusion, as mentioned before, a second blood sample was collected to help us determine the peak plasma levels for ketamine and its metabolites.

Immediately after the end of the infusion the cannula was removed in order to minimize any discomfort caused to participants. After the cannula was removed and our participants recovered from any side-effects produced during the infusion, they were asked to complete the PSI which is designed to capture any dissociative effects that they might be experienced during the ketamine administration (Mason et al., 2008). The SNK-W (Subjective Well-Being under Neuroleptic Treatment Scale) (Vothknech et al., 2011) is also administered in order to examine the effects of an intravenous infusion of either ketamine or placebo, on the participants' well-being. Approximately 1.5 hours after the drug infusion we collected a third and final blood sample and then participants were invited to enter the MRI machine for scanning. When the scan was completed, the neuropsychological test battery was repeated in order to help determine any changes in mood and hedonic capacity caused by ketamine.

The table that follows contains a brief description of the pre, during and post infusion assessments that take place out of the MRI scanner.

Pre-infusion	**SHAPS (Snaith-Hamilton Pleasure Scale)**	This 14-item, self-administered, scale is used to measure the present state of anhedonia.
	SNK-W	A self-administered, 20 item scale designed to measure subjective well-being.
	Wechsler Memory Scale	This task is designed to measure working memory.
	RAVLT (Rey Auditory Verbal Learning Task)	This task evaluates short term auditory verbal learning and memory.
During the infusion (20 min after the start of the infusion)	**White Noise Tas**k	This task is designed to detect any auditory hallucinations produced by ketamine infusion.
	EEfRT	The task measures anhedonia.
Post infusion-Immediate	**PSI (Psychotomimetic States Inventory)**	This index is used to capture any psychotomimetic effects experienced during the drug infusion. It measures the intensity of 29 different symptoms.
	SNK-W	This scale is repeated in order to capture any changes in well-being.
Post infusion-120min	**SHAP**S	This scale is repeated in order to detect changes in anhedonia.
	SNK-W	The scale is repeated to assess any changes in subjective well-being.
	EEfRT	This task is repeated in order to evaluate the antidepressant effects of ketamine on anhedonia.
	Probabilistic Learning Task	This task measures response bias towards the most frequently rewarded stimuli.

Table 1. *The table contains a brief description of the pre, during and post infusion assessments that take place out of the MRI scanner and notes the time of their administration*

Note

The questionnaires and tasks administered during this study have been designed in order to provide us with a broad overview of the effects that ketamine could produce acutely but also 2h after its administration when the antidepressant effects of the drug are first detectable. The present thesis focuses on the delayed (2h post administration) effects of ketamine on anhedonia and AM recall and rumination as those would be captured by the MID and the VAMP tasks. As a result, in our analysis we have only included data from out-of-the-scanner assessments and questionnaires that are relevant to our study aims, namely the RRS, the SHAPS, the SNK-W and the PSI. The section that follows will provide more details on those questionnaires, however, for completion, when the study design was explained, we briefly described all the assessments and tasks administered during the study day.

1 Behavioural Assessments

1.1 The RRS (Rumination Response Scale)

The RRS was developed as a more direct and reliable measure of assessing rumination that is related to but not confounded by depression (Treynor et al., 2003). The scale consists of 22 items that measure the two aspects of rumination: brooding and reflective pondering. The RRS items are rated on a four-point scale as follows: “1- Almost Never”, “2- Sometimes”, “3-Often”, “4-Almost Always”. The scores of this scale range between 22 and 88 points with higher scores indicating more frequent and intense ruminative thinking.

In our study the RRS was administered during the screening visit in order to give us a quantitative measure of ruminative thinking that our participants experience especially when feeling sad and depressed. The scale can be found Appendix C.

1.2 The SHAPS (Snaith-Hamilton Pleasure Scale)

The SHAPS is an instrument developed for the assessment of hedonic capacity. It is a 14-item, self-report scale that has been specially designed to minimise cultural, gender and age biases in the evaluation of the individuals' ability to enjoy pleasant activities (Snaith et al., 1995). Each of the items on the SHAPS has a set of four response categories: "Definitely Agree", "Agree", "Disagree", "Strongly Disagree" with either the "Disagree" responses receiving a score of 1 and either the "Agree" responses receiving a score of 0. The SHAPS is thus scored as the sum of the 14 items so that total scores ranged from 0 to 14. A higher total SHAPS score indicates higher levels of present state of anhedonia.

This scale was administered twice during each study day and was used in order to measure anhedonia in our remitted depressed volunteers. Comparison of the scores between the ketamine and placebo sessions would help us identify and effects that ketamine and/or placebo might have on anhedonia. The SHAPS as was administered in our study can be found in Appendix E.

1.3 The SNK-W

The SNK-W (Subjective Well-Being under Neuroleptic Treatment Scale) is a self-administered scale developed to measure the clinical relevance of subjective well-being as a measure of illness, treatment experiences and overall life satisfaction among mentally ill patients (Vothknecht et al., 2011). In this study we have used the short version of the scale which consists of 20 statements (10 positive and 10 negative). The scale has 5 subscales measuring: 1. mental functioning 2. self-control 3. emotional regulation 4. physical functioning and 5. social integration. Each subscale consists of four questions. The total score for the SNK-W ranges between 20 (bad subjective experiences) and 120 (perfect subjective experiences). In scoring terms, 10 of the items of the scale are scored in reverse and they are equally distributed among the 5 subscales. The scale can be found in Appendix F.

Although our participants were not currently receiving any antidepressant treatment and did not experience any depressive symptoms this scale was administered three times (baseline, right after the infusion and 2h post infusion) in order to capture the participants' well-being at key time points during the study and help us identify and changes related to the drug administration or the delayed- 2h post infusion- effects of ketamine.

1.4 The PSI (Psychotomimetic States Inventory)

The is a self-administered, 48-item questionnaire that has been developed in order to assess psychotomimetic symptoms after administration of psychoactive drugs such as cannabis and ketamine. Individuals rate statements describing their current experiences from 0 (not at all) to 3 (strongly). The PSI has 6 subscales measuring delusional thinking, perceptual distortions, cognitive disorganisation, anhedonia, mania and paranoia (Mason et al., 2008). The PSI can be found in Appendix D.

1.5 Correlations of scale scores with fMRI data

As part of our analysis the scores from these scales were correlated with the fMRI data. These correlations were performed based on our hypothesis about the relationship between the effects that ketamine would have on behaviour and the changes that the drug might produce on brain activations, 2h after its administration.

1.6 MRI battery of tasks

The scan lasts 90 min in total. While in the scanner, structural scans are acquired from our participants, followed by a resting state scan and the administration of two computerized tasks as well as a spectroscopy scan. During the resting state scan volunteers are asked to lie as still as possible and stare at a fixation cross. The first task is the VAMP task during which statements from personal events are presented to the participants and they are given some time to think about these events and rate how positive, negative or neutral they feel thinking about these events now. This task lasts approximately 30 min and was especially designed for this study and in order to measure AM recall. In the second task, the MID task, participants are asked to react to a target stimulus in order to win a signalled reward. MRS (Magnetic Resonance Spectroscopy) data are then acquired for the ACC. Following sections provides more details on the VAMP and the MID task including how those tasks were modelled and analysed.

MR Acquisition and Pre-processing of MR images

All scans were acquired using a GE750 3 Tesla scanner and a 16 channel head coil. T1-weighted MPRAGE scans were used for the DARTEL template and consequent normalisation of the functional data. Functional scans were obtained using a T2* sensitive gradient echo planar imaging (repetition time [TR]= 2000ms, echo time [TE]= 30ms, flip angle= 75^0, slice thickness = 3mm, number of slices = 42). The initial four volumes for each timeseries were discarded in order to minimise non-steady-state effects.

All functional and structural data were analysed using SPM-12. Standard pre-processing steps were used for the fMRI data and included: a. slice timing correction to account for the temporal differences between the acquisition of the slices b. realignment of the functional volumes using rigid body transformations, involving rotations and translations along the x, y, z axis in order to align each volume with a reference volume c. co-registration of the functional images of each participant to the structural ones from the same individual, to provide more precise spatial localization of any signal changes that might be revealed during the analysis d. normalization of all the functional data with the use of a DARTEL template.

The DARTEL (Diffeomorfic Anatomical Registration Through Exponentiated Lie algebra) template is created from grey matter and white matter segments acquired from the structural scans of the participants, and therefore, it is unique for a specific data set (Ashburner, 2007). This template allows for improved characteristics of brain shapes and a more precise inter – subject alignment. We did not expect ketamine to produce any changes in brain anatomy, nor did we expect significant anatomical changes to occur within our subjects between the two study visits thus we chose to use the structural scans from the participants' first session as the segments that were used to construct the template. The same template was used for the analysis of all functional data.

Study Tasks

The VAMP and the MID task are the main focus of our study. In the following sections we will provide more details on how those tasks were modelled and analysed. Moreover, since the VAMP was specifically developed for this study, we will also describe the steps that lead to its development.

2 General 1st level and 2nd level modelling

Prior to examining the effects of ketamine's delayed action on the MID as well as the VAMP task, each participant's functional time series required modelling according to the experimental conditions of each task. The standard approach for fMRI data, is to model, within each subject, the activations of each individual voxel in isolation using the GLM (General Linear Model) approach. In this way a design matrix is created for each individual, which incorporates all the task manipulations (task-related conditions) as well as potential nuisance parameters such as motion (motion-related regressors), which could influence brain activity. The design matrix for each subject shows how much each regressors could account for the activations observed in each particular voxel during each experimental task condition.

In order to examine, at an individual subject level, the differences in the BOLD signal between the task-related regressors, t-contrasts, are created which allow us to compare the task-related conditions to each other and identify statistically significant changes between conditions. At a group level, these contrasts are compared to each other using appropriate statistical testing in order to identify whether there are significant changes between different experimental conditions for the whole group or between the ketamine and placebo sessions -2nd level analysis. The results of the 2nd level analysis could identify significant clusters of brain voxels in which the activation between the different experimental conditions and/or between the two drug sessions is significantly different and thus allows us to identify brain areas which are important for the task manipulations but are also potential targets for the antidepressant effects of ketamine.

3 The MID (Monetary Incentive Delay) task

The version of the MID task used in this study is mostly comparable to the one used by Knutson and colleagues 2001 (Knutson et al., 2001). Prior to performing the task in the scanner, both during the screening visit as well as during the actual study days, volunteers were trained for this task. Training for the MID involves the presentation of a cue which participants learn to associate with a specific monetary reward. In the scanner, and in order to win the monetary reward, participants need to press the left button of their button box as fast as they can, every time they see a white square on the screen. Before the white square appears, one of the three cues that are part of this version of the task is presented. The cues consist of: a circle with one line which signifies that participants could win £0.20 (Low_win trial). A circle with two lines, which signifies that participants could win £2.00 (High_win trial) and a triangle which means that participants do not win anything (Neutral trial) but they still have to respond to the white square when it appears on the screen. Sometimes, an "X" appears on the screen and participants are told to not make any response (Passive_Trial). Figure 2 shows how the task is shown in the scanner.

In each trial of this task, different delay intervals exist between the presentation of the cue and the presentation of the white square. During that delay period the fixation cross appears on the screen. Participants win the monetary reward, signalled by the cue, only if they respond fast enough, by pressing the left button, when they see the white square. At the end of each trial a written statement informs the volunteers whether they won or not. If participants press the button during the fixation cross- premature responses-or a maximum of 100ms after the presentation of the square, the trial will register as unsuccessful. In this study, a total of 96, randomly arranged trials were presented. In order to ensure that there is no learning of the trial sequence, two different playlists were created and were presented in a random order, on each study day.

3.1 Modelling the MID task

For the purpose of better understanding as well as modelling the task for analysis, we can divide each trial of the MID into three phases. First, during the anticipation phase, the visual cue (circle or triangle) appears which represents the valence of the forthcoming reward. The presentation of the cue during that phase of the MID elicits motivational salience and is followed by an interval period of variable length during which the fixation cross appears on the screen. Afterwards, the target (square) initiates the participants' behavioural response in order to obtain the reward that was signalled during the anticipation phase (second phase). Finally, during the feedback phase, participants learn whether they have obtained or lost the expected reward. During the anticipation phase, the visual cue could signify the delivery of a big or smaller reward as well as a neutral outcome where no win occurs. As a result, by contrasting the anticipation and feedback phases of task trials where a monetary reward could actually be obtained, with the corresponding task phases of a neutral trials we can identify the brain areas that are important for winning or not, a big or smaller reward.

3.1.1 On an individual subject level

On an individual subject level, three conditions were defined for the anticipatory phase of the task –"High_win", "Low_win" and "Neutral"-according to the cue (circle with one line, circle with two lines, triangle, respectively) that was presented to the participants. For each of these task conditions the duration of the anticipatory phase included the cue presentation but also the delay interval during which the fixation cross remained on the screen (phases one and two, as mentioned in Section 2.1). Since the duration of the delay interval is variable, the duration of these conditions also varied and was between 4050ms and 4400ms. The response to the target square was modelled as a separate task condition. Depending on how fast participants responded to the target the duration of this condition was between 150ms to 350ms. Finally, five conditions were created for the feedback phase.

Two conditions were created to model the successful trial outcome of the expected high –"High_win_succ" -and low –"Low_win_succ"- reward. Another two were used to model the unsuccessful outcome of high- "High_win_unsucc"-and low- "Low_win_unsucc"-reward trials. Finally, a neutral condition "Neutral_feedback" was created for the feedback phase of those trials where no win was expected. The feedback phase conditions had a fixed duration of 1450ms. Finally, the passive trials were modelled all together as one condition with a duration of 4250ms. Missed trials, where participants failed to make any response, were also modelled as a separate condition. In this case, the three phases of this trial were modelled as one condition with a total duration of 10secs. If more than 10% of the total number of trials were missed on each scanning visit the data from this visit was excluded from the analysis.

3.1.2 On a group level

Reward anticipation and feedback for all the successful trials was the primary contrast of interest of the MID task in the group level. However, analysis of the level of reward (reward magnitude) on both anticipation and feedback was also examined. Specifically, for the analysis of the anticipation phase of the MID, the "High_win" and "Low_win" conditions were contrasted with the "Neutral" condition and in order to examine the role of reward magnitude, the "High_win" and "Low_win" conditions were compared to each other. For the analysis of the feedback phase of the MID trials, the "High_win_succ" and "High_win_unsucc" as well as the "Low_win_succ" and Low_win_unsucc" conditions were compared with the "Neutral_feedback" condition. The role of reward magnitude during feedback was examined by comparing the " High_win_succ" with "Low_win_succ " conditions with the "High_win_unsucc" and "Low_win _unsucc" conditions, respectively.

In order to examine which brain areas are important for each phase of the task, the placebo session was analysed separately, and a one-sample t-test was performed for each condition of interest. Comparisons between the ketamine and placebo sessions were made using a paired t-test that allowed us to investigate the delayed effects of ketamine administration (2h post infusion) on the brain areas that are activated for each condition of the MID task. Finally, the effect of session was examined by isolating data from participants who received ketamine in their first session and comparing them to the first session placebo data. This analysis was performed using a two sample t-test.

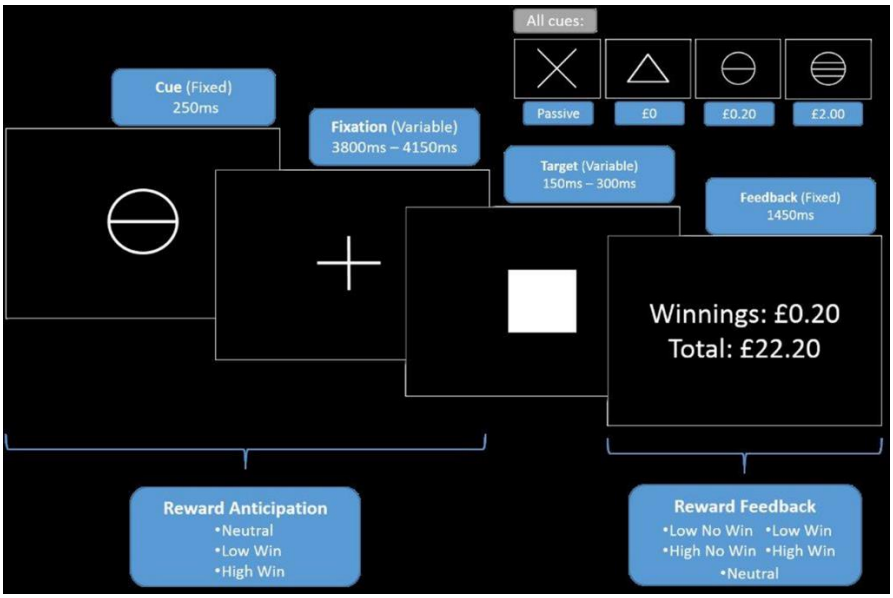

Figure 2. *The MID task has been designed specifically for fMRI studies. The design of the task allows the examination of the anticipation and feedback phases of the task separately.*

3.1.3 Correlations with behavioural data

The participants' performance to the SHAPS, before the administration of ketamine or placebo, was used as a regressor in order to explore whether baseline anhedonia would correlate with the brain activations observed during the MID, under placebo. Moreover, the difference (Delta) score between the baseline SHAPS scores and those obtained when the scale was completed, 2h after the ketamine administration, was used as a regressor in our analysis. The aim was to explore whether changes in anhedonia would correlate with changes in brain activity observed between the ketamine and placebo sessions for the anticipation and feedback phases of the MID. In addition to the SHAPS, anhedonia is also measured acutely, at the end of the drug infusion, with the PSI which contains an anhedonia related subscale. The anhedonia scores captured by the PSI were also correlated with the changes in brain activation produced by ketamine, compared to placebo.

3.1.4 Region of Interest Analysis

In addition to examining the MID task at a whole brain level, ROIs (Regions of Interest) were identified *a priori* and were used for a more targeted exploration of the role that these brain areas might have during reward anticipation and feedback for the MID task. These ROIs were derived from the literature around reward processing as well as previous fMRI studies that have examined the MID task. The activations in these regions were examined after placebo administration and were compared between the ketamine and placebo sessions. The effect of session in the activation of these ROIs, during different contrasts, was also explored. In the sections that follow, we describe how those ROIs were selected, created and used in our data analysis.

3.1.4.1 Striatum

The striatum is located in the forebrain and it is the largest anatomical structure and main input module of the basal ganglia. The striatum receives glutamatergic input from many brain areas including the cerebral cortex and other parts of the limbic system such as the amygdala, hippocampus and thalamus. Moreover, the striatum receives ascending dopaminergic input form the midbrain. It can be anatomically subdivided into the ventral striatum and dorsal striatum. The NAc (Nucleus Accumbens) and the olfactory tubercle form the ventral striatum whereas the dorsal striatum comprises of the putamen and the caudate.

The striatum is a critical component of the motor system and it plays a central role in reward processing and cognition (Wilson et al., 2018). Specifically, the ventral striatum is mainly involved in reward processing, reinforcement and motivational salience, whereas the dorsal striatum is mainly involved in cognitive processing (O'Doherty, 2004). There is evidence indicating that striatal function in relation to reward processing could be altered in depression (Pizzagalli et al., 2008). Moreover, due to the glutamatergic input that the striatum receives, activations in that brain area could also be modulated by ketamine administration (Malhotra et al., 1998).

In order to investigate the role of the dorsal striatum during the MID task and examine how ketamine might alter the activations in that region we created probabilistic, bilateral and unilateral,ROIs for the putamen (dorsal striatum) and the caudate (dorsal striatum) separately as well as the NAc. The definition of these ROIs was according to the FSL Harvard-Oxford atlas. The probabilistic ROIs created for the dorsal striatum brain areas were thresholded at 0.20 and binarized using the *fslmaths* tool as implemented in *fslutils* (Jenkinson et al., 2012). A bilateral and unilateral ventral striatal ROI (including the NAc) was also defined (Montgomery et al., 2006).These ROIs were also combined with SPM probabilistic grey matter mask (threshold 0.20) to ensure that no areas extending into non-grey matter were included.

3.1.4.2 VTA (Ventral Tegmental Area)

The VTA comprises of a group of neurons located in the midbrain. From the VTA originate the dopaminergic neurons of the mesocorticolimbic dopamine pathway. These mesocorticolimbic dopaminergic neurons are implicated in the brain's reward circuitry. Moreover, the VTA plays an important role in cognition, motivation as well as emotional processing (Lisman and Grace, 2005) . Neurons from that brain area project to the prefrontal cortex and the brain stem as well as several brain regions in between. The VTA ROI used for this analysis was based a study examining VTA activity during resting state (Murty et al., 2014). Bilateral as well as unilateral VTA ROIs were created and the activation of that brain area was examined for all the contrasts of interest.

3.1.4.3 Amygdala

The amygdala is a set of nuclei which is located deep and medially within the temporal lobes of the brain. Research has shown that this brain region which is part of the limbic system, is important for memory, decision making as well as emotional responses (Kensinger, 2009).The amygdalae share direct connections with the ventral striatum and the VTA and are sensitive to dopaminergic modulation during reward processing. Moreover, the connectivity and activation of the amygdala appears to be altered in depressed as well as remitted depressed individuals (Young et al., 2014, Young et al., 2016).A bilateral amygdala ROI was defined using the FSL Harvard-Oxford subcortical atlas. The role of the left and right amygdala was also examined, using ROIs based in the FSL Harvard-Oxford atlas.

4 The Valenced Autobiographical Memory Paradigm

4.1 Task Development

The VAMP is a novel personalised autobiographical memory task that was designed and developed specifically for this study and in order to be used in the MRI scanner. Inspiration for the design of this task was drawn from the classic version of the AMT (Autobiographical Memory Test) as well as from another task that was used in a study by Nicholson and colleagues (Nicholson et al., 2016) and which examined AM recall in conversion disorder.

In the classic version of the AMT which was developed based on methodology by Williams and Broadbent (1986), participants are presented with a series of cue words, for which they are asked to produce a specific memory. The memories provided are then classified according to their level of specificity. Different versions of the AMT have been employed across different studies and these versions vary in the number of cue words presented to participants as well as the emotional valence of the words used for the task which could be positive, negative or neutral (Liu et al., 2013). Several studies have also used prompt words, which are presented to participants when they fail to produce a specific memory after a cue word is presented. Prompting is supposed to increase the specificity of AM retrieval. Finally, different versions of the AMT provide participants with different time windows during which they could recall a specific memory. The length of these windows ranges between 30 secs to 60 secs whereas there are some studies in which recall time is not at all restricted and participants are free to set up their own pace for this task (Griffith et al., 2012).

The AMT is a well validated task and has been successfully used to capture deficits in the specificity of AM recall in depressed patients. It has also been used in remitted depressed volunteers as well as individuals with a high risk of developing depression (Liu et al., 2013). The task focuses on the number of AMs recalled and their specificity, these are the main outcomes of the task and are used to assess AM. Although in depression overgeneral AM persists during remission (Warne et al., 2019), and thus we expected it to be also present in our group of volunteers, ketamine's delayed effects have not been linked directly to improved AM retrieval. We hypothesized, thus, that any positive effects that ketamine might produce on AM recall facilitation would be reflected as changes in the activation of and the connectivity between brain areas that are important for AM and not on

the number and/or the specificity of these memories. Moreover, we expected that ketamine would modulate brain activation differently, based on the valence of the AMs. As a result, the use of cue words and prompts to generate recall of positive, negative and neutral AMs, as in the AMT, needed to be adjusted to our specific needs.

The use of fMRI in our study, and the complicated statistics that are involved in fMRI data analysis required the development of a task that consisted of several repetitions of a specific condition in order to provide us with enough statistical power. Moreover, in order to capture any effects of ketamine on brain activation, we needed a task that would provide participants with sufficient time to recall and ruminate on specific AMs. The fact that we use a cross-over design with the same volunteers receiving both ketamine and placebo during two separate sessions, made it necessary to design a task during which the same memories would be recalled on both sessions. That would ensure that any changes in brain activation produced by the drug and identified during the task would be due to the effects of ketamine and not the result of the difference in the valence and quality of memories recalled on each session.

In order to control for the valence of memories that participants would be asked to recall both between session but also between participants we drew inspiration from a study conducted by Nicholson et al. (2016). In this fMRI study participants with motor conversion disorder were scanned while presented with blocks of statements (1block = 8 statements) for three different event types that were classified as “severe” using the LEDS (Life Events and Difficulties Schedule). At the end of each block, participants were asked to rate how upsetting thinking about each specific event has been for them. Although in the Nicholson study participants were not provided with sufficient, for our purposes, time to think about the events, the use of a semi-structured interview such as the LEDS in order to interview the participants and rate the severity of their experiences would ensure that equally severe events, for all participants, would be used during the task. As a result, the LEDS interview was adapted to the demands of our study and fMRI task. The interview can be found in Appendix B. Moreover, rating of the valence of the event at the time of the scan, also implemented by Nicholson et al. (2016) and would allow us to capture any effects that ketamine might have on mood and anhedonia during the study day and would provide us with a measure of emotional valence of the retrieved AMs that we could use as a control factor in our analysis.

4.2 The LEDS interview

The LEDS interview is a well validated tool that has been used in order to provide a classification of the severity of life events that could sometimes be considered triggers for certain psychiatric conditions such as depression or conversion disorder. The interview is semi-structured and life experiences are classified as either life-events or difficulties (Brown and Harris). According to the LEDS a difficulty is an ongoing struggle that lasts for at least 4 weeks whereas a life event (it will be referred to as event from now on) has a much shorter duration. There could be several events within a specific difficulty. For the purposes of our study, and in order to ensure that participants could remember in detail their experiences during the limited time of the fMRI task we focused only on events and not difficulties.

According to the LEDS rating scale, negative life events could be classified in 9 different categories which are further subdivided into different subgroups. For example, category 4 focuses on "Money/Possession" related events and it encompasses a. financial crises/debts, b. financial gains, c. loss, damage threat to property etc. Each event is classified by the interviewer in one of those categories and then the events are rated according to their degree of unpleasantness. There are 4 degrees of 1. Marked unpleasantness 2. Moderate unpleasantness 3. Some unpleasantness and 4. Little or No unpleasantness. Each event receives a contextual rating which is based solely on the event, without taking into account the feelings of the person/persons involved, and a reported rating which takes into account the narrator's way of talking about that particular event.

The LEDS interview has been developed with a focus on negative life events that could trigger certain psychiatric conditions(Brown et al., 1988). However, a rating of positive events also exists and was used for the purposes of our task. Positive events, as defined by the LEDS, are always associated with symptom improvement in affective disorders. An ad hoc examination of cases where improvement was observed after a depressive episode, revealed that events which took place close to the time when a marked improvement in depressive symptoms was observed were all characterized by a "fresh start" for the individuals(Brown et al., 1988).

Positive events usually involve some action or change that led to a marked reduction of some difficulty and they always involved the respondent personally. Although this definition of a positive event might be sufficient for the LEDS, we believe that it could be rather restrictive for our participants who are not currently depressed. For that purpose, the definition of the positive events was adjusted and all events that were remembered as pleasant experiences by the individuals were included in our task provided that participants had a good recollection of the event and that they would still feel positive thinking about it. The same 4 point rating system that was used for the negative life events was also applied to the positive ones, with “marked” pleasantness to be the top rating for a positive event.

Neutral life events as well as a control condition were also part of our task. Life events could be classified as neutral when they score as ” 4. Little or no unpleasantness/pleasantness” in the LEDS rating scale. These events usually involved routines, such as going to work or doing the weekly shopping. These experiences were not considered to be sad or particularly exciting for both the narrator and the interviewer. The choice of an appropriate control condition for this task, was rather difficult. The specifics of fMRI analysis required that our control condition would have the same time length as that of the active task conditions but would also be emotionally neutral and engage thought processes. We chose a control condition that would require our participants to count silently and upwards, in odd numbers, every time starting from a different number. The LEDS interview as administered in this study can be found in Appendix B.

4.3 Administration of the VAMP

4.3.1 Preparation for the task- interview day

Preparation for this task started with the administration of the LEDS interview. After a successful screening visit and before they attended their first study session, participants were invited for an interview day. Before their interview and during the screening visit, participants had seen a short demonstration of the VAMP in order to familiarise themselves with the task. The LEDS interview was also explained to them in detail and before attending their interview day they received an email with detailed instructions around the events that would be suitable to the task. Specifically, participants were encouraged to talk about the most positive and negative life events that occurred within 12 months prior to taking part in the study. However, if they believed that something more severe, either positive or negative, had occurred in the recent past, they could go back up to 5 years and talk about that event. During the LEDS interview which was recorded participants were instructed to share as much contextual information as possible and that was essential for the preparation of the fMRI task.

4.3.2 Rating and Statement Preparation

The positive and negative events were rated by the interviewer as well as another independent rater and only those that score "1- Marked" in the LEDS scale were considered for the task. Since the task involves recalling autobiographical events the recollection of which could be potential unsettling for some of our volunteers a debriefing was offered, and details of counselling services could be provided if necessary. For the neutral events a rating of "4 Little or no unpleasantness" was necessary.

Participants were encouraged to talk about more than one positive, negative and neutral event. In the case of two or more events of the same emotional valence which also received the same LEDS rating, the level of contextual information recalled from the participants would determine which event would be used for the fMRI task. For each event, 20 matched-in-length statements were created and were split into two groups of 10 statements each. The two groups included different statements from the same event which provided us with a coherent narrative of the event.

The two groups of statements were presented to participants, one group on each study day, in a random order. The statements contained as much contextual information as possible while omitting the emotional components of the narrative, since these could influence the participants rating of the events. Furthermore, 20% of the statements were altered and the details they contained were inaccurate thus ensuring that participants would be paying attention to the statements that were presented to them in the scanner.

4.4 Scanning

Scanning took place 2hours after the intravenous infusion of ketamine or placebo and the VAMP task was performed right after the resting state scan. Since the VAMP task required great level of attention and it could also be emotionally challenging, we chose to split the task into two runs. During each run five statements from each of the positive, the negative and the neutral life events were presented in that order to the participants. Participants had 6 secs to read carefully each statement and decide whether the statement was true or false. If the statement was true to the event, then participants had 12 secs to recall and ruminate about that event in as much detail as possible. At the end of the 12 secs participants were asked to rate, using a VAS scale, how positive or negative they felt thinking about this event at the time of the scan. If the statement was false or participants failed to respond in time, the task automatically would move on to the next statement.

The control condition, which asks participants to count upwards, silently and in odd numbers, every time starting from a different number was presented in a random order, between statements, a total of 12 times during each run. That number (12 times) matches the total number of correct statements (positive, negative and neutral) that are presented to participants during each run of the task. A 6min structural scan followed end of the 1st VAMP run allowing our participants to rest before the task began again. During the 2nd VAMP run of the task different statements, from the same events, were presented while the order of the events was kept the same between runs and between participants. Each run could last up to 15min, but the actual duration of the VAMP task could vary depending on how many statements participants identified as false.

4.4.1 On an individual subject level

For the purpose of modelling the VAMP task for analysis we divided each statement presentation into three phases. The first phase is the decision phase during which participants are asked to read the statement carefully and decide whether it is "TRUE" or "FALSE". If the statement is identified as "TRUE", the retrieval phase follows, when participants are asked to think about the event that is presented to them in as much detail as possible. The rating phase is the final phase of the statement presentation and during that participants are asked to rate how positive or negative they feel thinking about this event at the time of the scan.

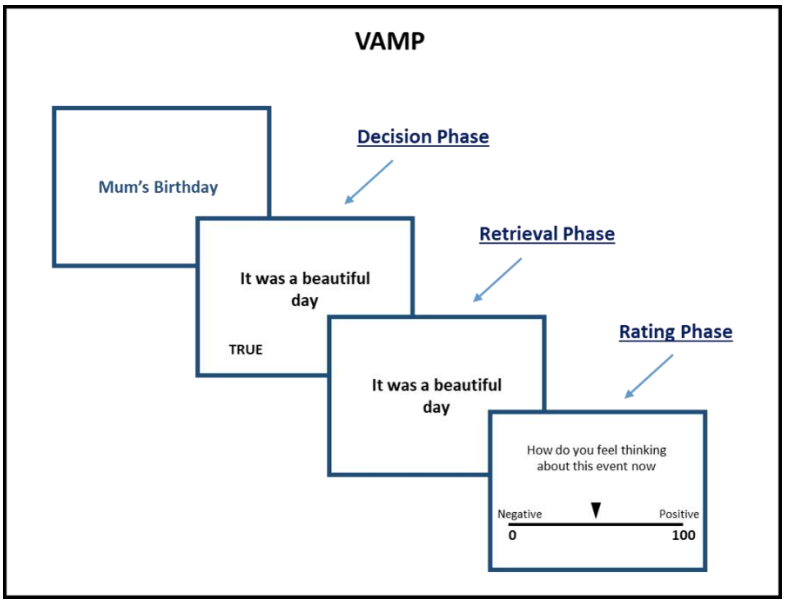

Figure 3. *The VAMP is a personalised AM task. The Retrieval Phase of TRUE statements is the one used for the analysis of the fMRI data from this task.*

Four conditions can be identified for the decision phase of the task a. True_correct, b. False_correct, c. True_incorrect and d. False_incorrect. All these conditions are 6 seconds in length and the first two represent statements that are actually true (a) or false (b) and have also been identified correctly as such by the participants. The last two conditions represent statements that have not been correctly identified as true (c) or false (d) by the participants.

During the retrieval phase of the VAMP two 12 secs long conditions are modelled. These conditions are: True_correct_retrieval and False_incorrect_retrieval. These two conditions represent the AM recall period for statements that have been identified as "TRUE". In the False_inocrrect_retrieval condition however, an incorrect decision regarding the statement was made and these conditions although part of the task model, are excluded from the analysis. The rating phase is also modelled with a separate condition that is called "Rating" and is 4 seconds long. Ratings that correspond only to True_correct conditions of the decision phase are part of the final analysis. The control condition is modelled as a whole and is 22seconds long. All these conditions are created separately for the positive, the negative and the neutral events. During the individual subject level analysis, the two runs of the task are modelled together in a single design matrix.

4.4.2 Group comparisons

The True_correct_retrieval condition is of particular importance for the analysis of the VAMP task. This condition would allow us to examine brain activity while participants are recalling AMs based on correct statements. On a group level and just for the placebo session, we compared the True_correct_retrieval condition between the positive, the negative and the neutral events to see whether the brain areas that are implicated in recall differ based on the valence of the retrieved memory. We also contrasted these conditions with the control condition to identify brain areas that are important for AM recall. All placebo group comparisons were made using a one-sample t-test. The True_true_correct retrieval condition was also compared for positive, negative and neutral memories between the ketamine and placebo sessions where a paired t-test was applied.

4.4.3 Correlations with behavioural data

The participants' scores on the RRS, were used as regressors in order to explore whether general rumination levels would correlate with brain activity in areas that are activated during AM recall. Moreover, the scores from the same scale were used to explore whether ketamine would modulate brain activity differently based on how much participants ruminate in general.

4.4.4 Connectivity of the VAMP task

Several cortical and subcortical brain areas have been identified, in the literature, as important for AM recall. Amongst these brain areas the amygdala, the sgACC and the PCC are not only critical for AM retrieval but have also been implicated in the AM deficits observed in depression.

In this study we used PPI (Psychophysiological Analysis) in order to examine the connectivity of these regions (seeds) with the rest of the brain. PPI is a task connectivity method that allows us to determine whether the correlation in activity between two distant areas is different under different task conditions. Specifically, this analysis examines whether there is an interaction between the physiological state of the brain during the task and the functional coupling between two brain areas, or between a seed region and the rest of the brain (O'Reilly et al., 2012).

We applied the PPI methodology in the VAMP task in order to identify:

1. In the placebo group, changes in the connectivity between our seed regions and the rest of the brain during positive and negative recall compared to neutral. For this analysis we examined the connectivity of our seed region during the retrieval phase of the positive and negative events and compared it to the retrieval phase of the neutral events.
2. In the placebo group, changes in the connectivity related to the emotional valence of the memory. For that purpose, we examined the connectivity between the seed region and the rest of the brain, during the retrieval phase of positive events compared to negative events.
3. Whether ketamine, compared to placebo, would produce changes between the connectivity of the seed region with the rest of the brain. For that purpose, we contrasted the retrieval phase of positive events with that of neutral events and

compared it between sessions (ketamine session vs placebo session). We repeated the same analysis for the negative event.

4. Whether ketamine compared to placebo would produce emotional-valence-specific changes in the connectivity of the seed region with the rest of the brain. For this analysis, we contrasted the retrieval phase of positive AMs with that of negative AMs and then compared the ketamine and placebo sessions.

4.4.5 ROIs for the VAMP connectivity analysis

4.4.5.1 Amygdala

The amygdala is a pivotal component of the affective network. This bilateral brain structure located deep in the medial temporal lobe in close proximity to the hippocampus. The amygdala could act as a hub in a wide range of emotional processing tasks. In patients with MDD as well remitted depressed individuals the amygdala appear overactive during AM recall but also emotional processing (Young et al., 2014, Young et al., 2016). The connectivity of the amygdala with the rest of the brain is also altered in MDD. Specifically, decreased connectivity between the amygdala and parahippocampal regions has been identified in depression along with increased connectivity between the amygdala and occipital/parietal regions, the hippocampus as well as the PCC (Young et al., 2016). A bilateral ROI was created for the amygdala based on the FSL-Oxford atlas and was used for the PPI analysis.

4.4.5.2 PCC

The PCC along with the angular gyrus and the amPFC (anteromedial Prefrontal Cortex) comprise the most commonly activated regions of the DMN. The PCC is a heterogeneous brain structure with subdivisions characterized by distinct patterns of structural and functional connectivity (Leech et al., 2011). In more detail, activations at the ventral PCC functionally correlate with the rest of DMN and that pattern of co-activation is present across all self-generate tasks, including those tasks that involve AM recall (Fan et al., 2019). The dorsal PCC has been linked to autonomic arousal and awareness. It has an important function in monitoring behaviourally relevant stimuli as well as environmental changes (Fan et al., 2019). Although they present with relatively distinct functions, the dorsal and the ventral PCC are anatomically interconnected and also present with strong connections with the precuneus (Leech et al., 2012). In our analysis we have examined the connectivity of the PCC as a whole, but also the connectivity of the dorsal and ventral PCC. Bilateral ROIs were created to examine the distinct roles of the dorsal and the ventral PCC. These ROIs were 6mm spheres that were created using MarsBaR.

The centre of these spheres ware located at the voxel that presented with peak activations for the ventral PCC (MNI 2 -58 28) and dorsal PCC (MNI 2 -34 40) in Leech and colleagues(Leech et al., 2011).

4.4.5.3 sgACC

The sgACC is located ventral to the genu of the corpus collosum and has been implicated in regulation of emotional behaviour Evidence from neuroimaging studies suggests that the area presents with increased connectivity with the amygdala and insula which could contribute to altered emotional processing in depression (Connolly et al., 2013, Drevets et al., 2008b). Moreover, increased connectivity between the sgACC and the middle and inferior frontal gyri significantly correlates with rumination in MDD (Connolly et al., 2013). According to its anatomical definition, the human sgACC consists of angular cortex characterised as BA (Brodmann Area)24 anteriorly and BA25 posteriorly. We have used the BA-FSL atlas in order to create the bilateral sgACC ROI for our analysis.

Behavioural data

1 Introduction

Ketamine as a psychoactive drug with antidepressant effects (2h-24h post administration) could influence the emotional state and behavioural characteristics of individuals. Remitted depressed volunteers, like the cohort recruited for this study, could theoretically be even more susceptible to the behavioural effects that ketamine might produce, compared to healthy, never depressed volunteers, since these individuals, although asymptomatic at the time of the study, might still present their brain activations might present with some of the neural correlates of increased rumination and anhedonia (Berman et al., 2011, McCabe et al., 2010). In this chapter we will present the general characteristics of our study sample including age and gender balance. We will also examine the levels of ketamine and its major metabolite, norketamine, at the end of the 40min infusion as well as 2h post infusion. Recently, HNK (Hydroxynorketamine), another active metabolite of ketamine, has gained a lot of interest in the literature as a potential mediator of the drug's antidepressant action (Zanos and Gould, 2018). In our study we have measured the levels of HNK in order to investigate how its concentration changes over the course of the study.

The changes that ketamine might produce in general mood and anhedonia, acutely as well as 2h after its administration, are captured by the scales and questionnaires administered in the study (all the questionnaires can be found in the Appendices). In order to provide us with an understanding of how much our participants engage in rumination, especially when they feel sad or depressed (Treynor et al., 2003), the RRS is administered during the screening visit (see Appendix C). On the study days, the SHAPS (Snaith et al., 1995) is administered twice, at the start of the day and 2h after the drug infusion (for the scale see Appendix E). This scale was included to capture anhedonia symptoms that our participants might experience and help us determine whether ketamine would produce any significant changes in the SHAPS score. Additionally, the SWN-K scale is administered at three time points during the study day - at the start of the day, immediately after the drug infusion and 2h post infusion- in order to measure participants' baseline well-being and any significant changes that could be the result of the infusion and/or the delayed effects of ketamine (for the scale see Appendix F).

Shortly after the end of the drug infusion, and once they have recovered from any side-effects, participants were asked to complete the PSI, along with the SWN-K. This scale has been designed specifically to capture the dissociative effects of psychoactive drugs, such as ketamine and cannabis (Mason et al., 2008). In our study, the PSI was administered in order to measure the dissociative effects that ketamine produces acutely and would be experienced by our volunteers during the 40 min steady-state intravenous infusion (the scale can be found in Appendix D.

2 Results

2.1 General Sample characteristics

For the purpose of our study, we recruited a total of 36 male and female volunteers over approximately 36 months. In order to take part in the study, participants needed to have experienced at least one depressive episode in the past. At the time of the study however, our volunteers were in full remission from depression and free from any antidepressant treatment for at least three months prior to their involvement in the study. Their previous mental health history as well as their current mental health state were assessed using the M.I.N.I which was administered by the study doctor during the screening visit. A total of 150 volunteers were screened for our study. Approximately 2/3 of them still experienced significantly low mood, as was reported during the screening, and were thus excluded from the study. Moreover, MRI incompatibilities along with the multiple visits and the rigid timelines for the study were the main reasons that lead to exclusion from the study.

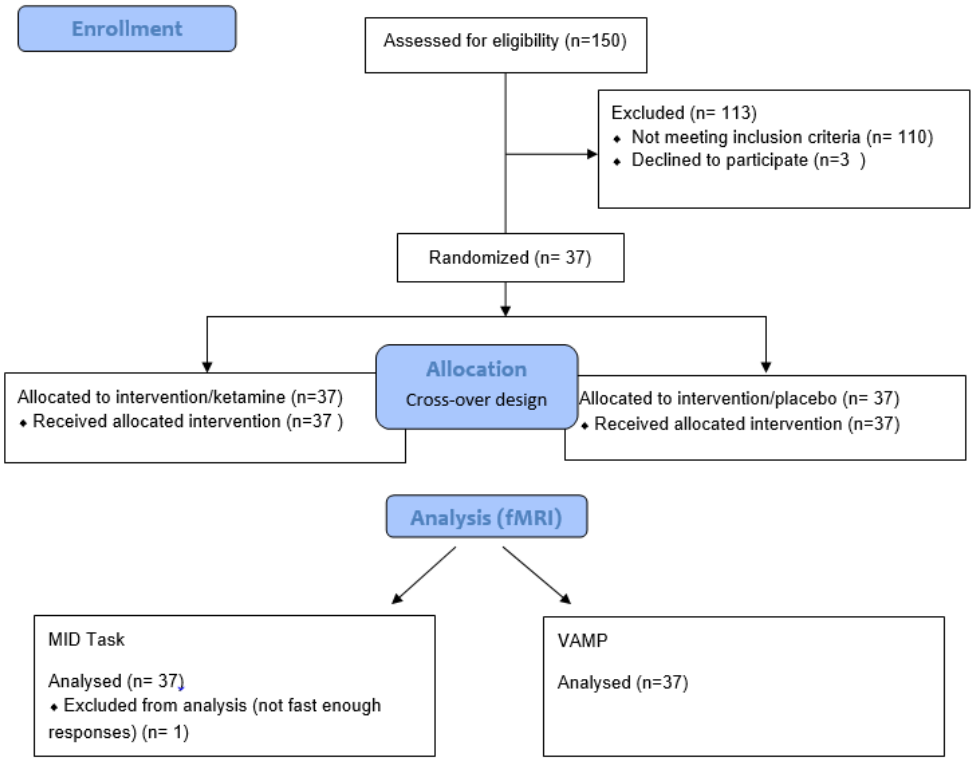

***Figure 4**. The CONSORT diagram shows how many participants, were screened, randomised and finally analysed for this study.*

2.2 Rumination

The RRS scale was administered once during the screening visit. Table 2 shows the average rumination scores in our cohort, as captured by the scale. The table also includes the age and gender balance in our sample. The range for RRS ratings is between 22 and 88 and the mean score for our sample is 44.81 (SD, ±14.31).

	Male (42%)		**Female (48%)**	
	Mean	SD	Mean	SD
Age	28.8	±9.10	28.3	±7.3
RRS	45.7	±14.36	44.29	±14.69

Table 2. *The mean age and RRS scores in our cohort. A two sample t-test did not reveal gender significant ($p<.05$) differences in rumination.*

Previous research has shown that there is a gender difference in rumination in the healthy population with females demonstrating higher levels of ruminative thinking compared to males. According to Nolen-Hoekseman who developed the RRS scale, the mean scores for healthy males and females significantly differ, with males scoring 39.6 and females 42(Nolen-Hoeksema et al., 1999). In our sample of remitted depressed volunteers there are no significant differences in the rumination scores between male and female participants. Moreover, the total average RRS scores as well as those obtained individually for males and females are numerically higher than the general average score and gender specific scores reported in the healthy population, suggesting that this group continue to ruminate.

2.3 Performance on Memory Tests

The scores on the RAVLT and Weschler memory test are also included in a separate table for each of the two study visits. These memory assessments are administered at the start of each study day to provide us with a measure of cognitive function, specific to memory encoding and recall at baseline

	RAVLT				**Weschler Memory Scale**	
	R1	R2	R3	DR	IM_R	DEL_R
Ketamine Session	7.77 (±1.59)	11.08 (±1.96)	12.69 (±2.3)	11.86 (± 2.70)	16.32 (±3.88)	18.47 (±3.48)
Placebo Session	7.6 (± 1.85)	10.8 (±2.48)	12.37 (±2.18)	11.63 (± 2.54)	15.83 (±5.03)	17.97 (±4.94)

Table 3. *The immediate and delayed recall measures of the RAVLT and Weschler Memory Scale did not reveal significant differences in the cognitive performance of our cohort between sessions (Paired t-test, p>.05).*

Performance in the RAVLT and Weschler memory scale did not change between the study sessions (Paired t-test, p>.05). Moreover, no effect of session was identified between those participants who had ketamine on their first session compared to those who were given placebo on their first session (Two sample t-test, p>.05). Both the RAVLT and the Weschler memory scale have an immediate and delayed recall component. In Table 3 the average scores of the three immediate recall phases (R1, R2, R3) of the RAVLT as well as the average score of the delayed (DR) recall of this scale are presented. The average scores from the immediate (IM_R) and delayed (DEL_R) recall component of the Weschler memory scale are also included.

2.4 Measures of Anhedonia and Subjective Well-being

2.4.1 Anhedonia

The self-administered SHAPS was completed twice on each study day, at the start of the day in order to provide us with a baseline measurement of anhedonia and 2h after the infusion, in order to capture any effects that either the ketamine or the placebo administration might have on anhedonia. The total SHAPS scores from both the ketamine and placebo sessions are shown in Figure 5.

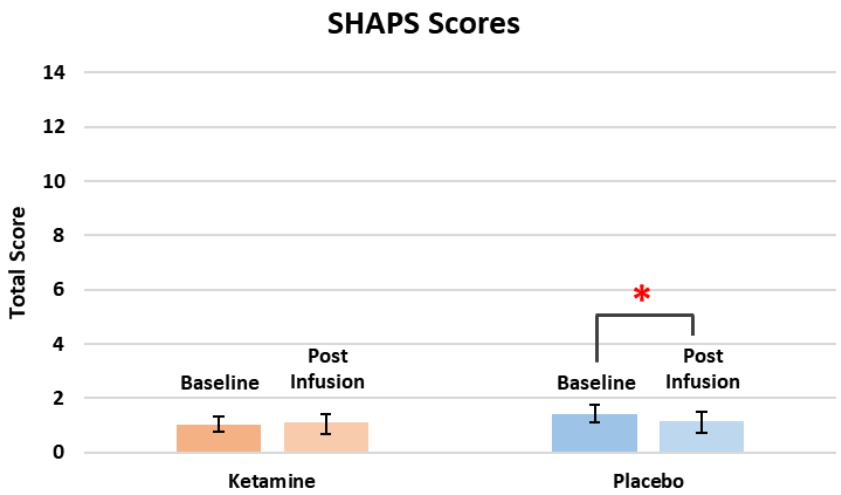

***Figure 5**. Anhedonia did not change 2h after ketamine (Wilcoxon Signed Rank test, p>.05). In the placebo session a significant reduction in anhedonia was identified (Wilcoxon Signed Rank test, p<.05)*

The total scores obtained at baseline and 2h post infusion were compared, separately for each session. The scores of this questionnaire were not normally distributed and thus non-parametric testing was used. We found that for the placebo session, there was a significant decrease in anhedonia 2h post infusion, compared to baseline (Mean baseline score= 1.47, SD = ± 1.94, Mean Post infusion score = 0.79, SD = ± 1.39, Wilcoxon Signed Rank test, p<.05). No significant changes were identified for the scores obtained during the ketamine session (Mean baseline score= 1.03 SD= ±1.94, Mean Post Infusion score = 1.11, SD= ± 1.85, Wilcoxon Signed Rank test, p>.05) and no significant changes were identified between the baseline scores of the ketamine and placebo days (Wilcoxon Signed Rank test, p>.05). Moreover, the change between the baseline and post-infusion scores was calculated and these differences were compared between ketamine and placebo. No significant changes were identified.

Finally, the effect of session was also examined and no significant changes in anhedonia were found between the baseline and post infusion scores of those participants who received ketamine on the 1st session compared to those who received placebo (Wilcoxon Signed Rank test, p>.05). The absolute scores of the SHAPS as well as the difference in the scores between baseline and placebo were entered into a correlation analysis with the brain activations in ROIs that are important for reward processing during the MID task. No significant correlations were identified.

2.4.2 Subjective Well-Being

In this study we have used the SWN-K in order to obtain a measurement of the participants' self-reported well-being at the start of each study day, immediately after the drug infusion and the 2h post the infusion. The mean scores from the SWE were for ketamine (Mean Baseline score = 95, SD = ±14,87, Mean Post Infusion score = 90.29, SD = ±12.88, Mean 2h Post Infusion score = 95.07, SD = ± 13.44) and for placebo (Mean Baseline score = 98.71, SD = ±12.1, Mean Post Infusion score = 97.79, SD = ±10.96, Mean 2h Post Infusion score = 97.36, SD = ± 9.52) are normally distributed and a multiple regression analysis was used to identify any significant effects of treatment (ketamine vs placebo) and/or the time that the scale was administered (start of the day, right after the infusion, 2h post infusion). Figure 6. shows the total scores from each timepoint for both the ketamine as well as the placebo session.

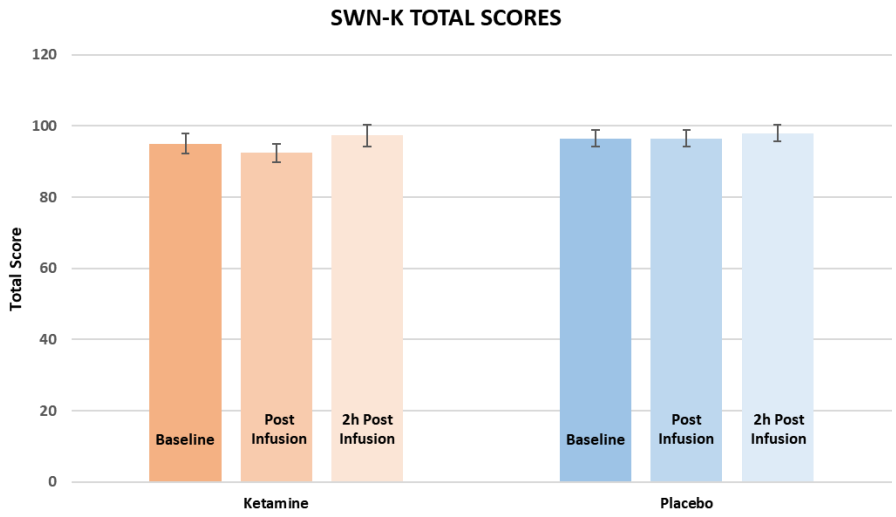

Figure 6. *Multiple regression revealed a significant effect of treatment in the ketamine session (F (23,1) =8.11, p<.05) but the effect of treatment over time was not significant (F (23,1) = 0.69, p>.05)*

Overall, there was an effect of ketamine with generally lower scores for the SWN-K during the ketamine session compared to placebo. This effect appeared to be driven mainly by the much lower scores obtained for this scale right after the ketamine infusion. When we examined the effects of treatments vs time, however, we did not identify any significant interactions and thus we did not explore this further. Finally, we did not identify any significant effects of session for the scores of this scale and there were also no significant changes between the baseline scores of the ketamine and placebo sessions (Two sample t-test, $p>.05$).

2.5 Ketamine: Pharmacodynamics and the dissociative effects of the drug

2.5.1 Ketamine Blood levels

The levels of ketamine, norketamine and the two isoforms of HNK ((2S,6S)-HNK and (6R,6R)-HNK)) were measured from a blood sample that was taken 5min before the end of the i.v infusion as well 2h after the ketamine administration. Seven participants were not included in this analysis since they reported intense feelings of dizziness and discomfort towards the end of the ketamine infusion and therefore no blood sample was collected at that time. In Figure 8 the missing values (gaps in the diagrams) represent metabolites whose levels were below detection in the samples. Analysis of the plasma levels from the collected samples showed that the average concentration of ketamine reduces considerably 2h post the infusion whereas the norketamine levels are stable . HNK levels increase 2h post infusion. Figure 7 show the average values of ketamine and its major metabolites at the end of the infusion and 2h post infusion. Figure 8. Shows the individual levels of those metabolites from all the participants and Table 4, provides the average values of ketamine and its metabolites at the end of the 40min i.v infusion and 2h post infusion.

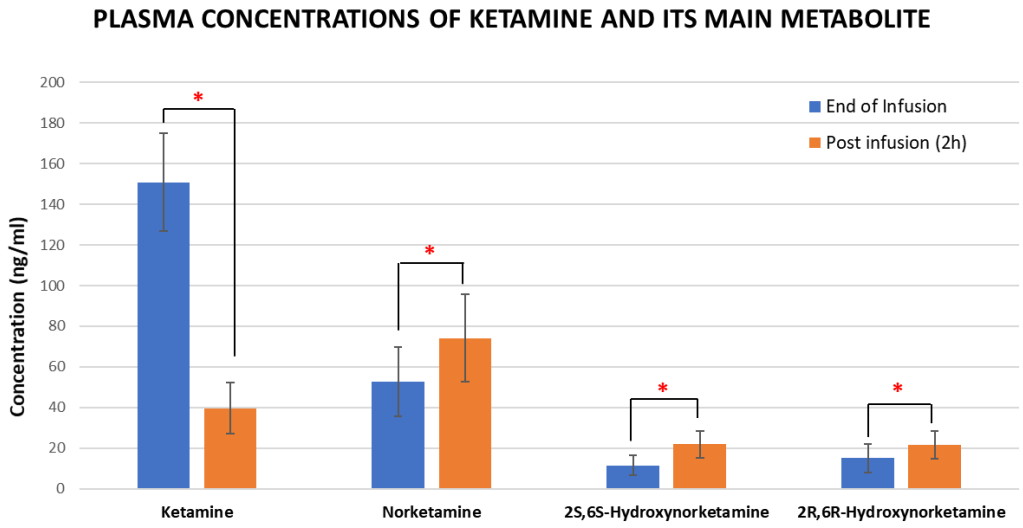

Figure 7. *The average values of ketamine and its major metabolites at the end of a 40min i.v infusion and 2h post infusion. Significant changes were identified for all metabolite levels 2h after the drug administration (Paired t-test., p<.05)*

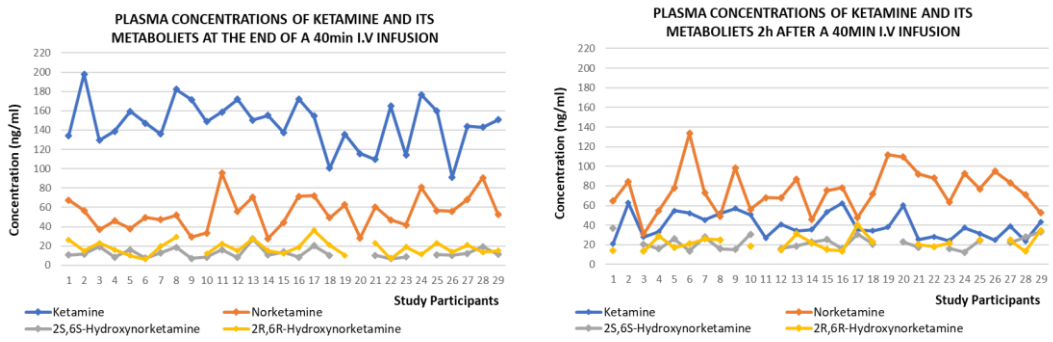

Figure 8 *Individual levels from ketamine's metabolites at the end of a 40min i.v infusion and 2h post the ketamine administration. Missing values represent participants where the metabolite levels were below detectable concentrations.*

	Ketamine	Norketamine	2S,6S-Hydroxynorketamine	2R,6R-Hydroxynorketamine
End of infusion	146.6 (SD ± 24.7)	54.74 (SD ± 17.49)	12.81 (SD ± 5.27)	18.64 (SD ± 7.47)
2h post infusion	39.9 (SD ± 12.78)	75.88 (SD ± 22.24)	22.51 (SD ± 6.73)	21.87 (SD ±7.24)

Table 4*. The levels of ketamine's metabolites at the end of a 40min i.v infusion and 2h post the ketamine.*

2.5.2 PSI scores

On each study day and at the end of the 40min drug infusion, participants were asked to complete the PSI. The scale is self-administered and higher scores on this scale indicate that participants experience more intense dissociative and psychotomimetic effects during the drug administration. After marking the PSI, the total score as well as the scores for the six subscales were calculated and compared between the ketamine and placebo sessions using paired t-tests. As expected, participants scored significantly higher on the PSI after a ketamine infusion, compared to placebo (Mean ketamine score= 48.36 SD= ±22.01, Mean placebo score= 15.10, SD= ±10.05). When we compared the scores for the six subscales of the PSI between the ketamine and placebo sessions, we found significantly higher scores under ketamine compared to placebo for all subscales (Paired t-test, $p<0.05$). The graph below shows the total score as well as the average score for the six subscales.

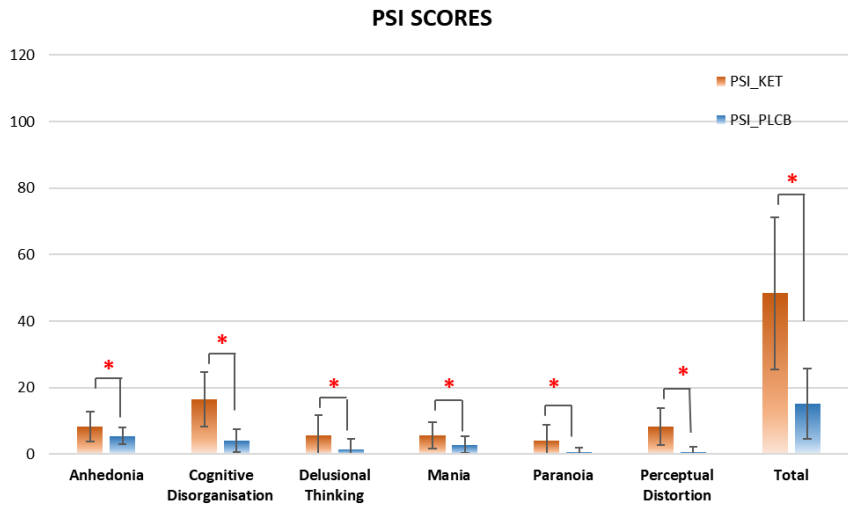

***Figure 9**. Ketamine produced significant changes in the PSI scores compared to placebo. Significant increases were identified for the six subscales, including anhedonia (Paired t-tests, p<.05)*

	Anhedonia	Cognitive Disorganisation	Delusional Thinking	Mania	Paranoia	Perceptual Distortion	Total
Ketamine	8.33 (SD ± 4.56)	16.47 (SD ± 8.07)	5.63 (SD ± 6.01)	5.57 (SD ± 3.95)	4.01 (SD ± 4.71)	8.37 (SD ± 5.56)	**48.4** (SD ± 22.92)
Placebo	5.43 (SD ± 2.48)	4.10 (SD ± 3.43)	1.47 (SD ± 2.99)	2.77 (SD ±2.51)	0.66 (SD ± 1.37)	0.67 (SD ± 1.47)	**15.1** (SD ± 10.55)

***Table 5**. The mean PSI scores for each of the subscales for the placebo and ketamine session.*

Note

The PSI was completed at the end of the 45min infusion and once participants have recovered from any side effects that they might have experience during the infusion. While filling in the PSI, participants were asked to imagine how they felt during the infusion and when any effects that the drug produced were most prominent.

3 Discussion

In this chapter we examined the general characteristics of our study sample, we looked at ketamine and its metabolites immediately after the infusion as well as 2h post infusion and we also explored the acute and delayed (2h post administration) effects of ketamine in our volunteers. The PSI was used in order to capture the dissociative and psychotomimetic effects that ketamine could produce during the infusion, while the SHAPS and SNK-W would help us understand how anhedonia and subjective well-being change over the course of each study session (ketamine vs placebo). The RRS scale provided us with a baseline measure of how much our participants engage in rumination, especially when they feel sad or depressed.

3.1 Ketamine and its metabolites

Ketamine, after its administration, undergoes extensive metabolism, via nitrogen demethylation, to norketamine (Zanos et al., 2018). This reaction is catalysed by the liver enzymes of cytochrome P450 and norketamine is further metabolized to DHNK (dehydroxynorketamine) and hydroxynorketamine (HNK). According to the pharmacodynamic model for ketamine's metabolism in patients diagnosed with unipolar and bipolar depression, norketamine, DHNK and (2R,6R;2S,6S)-HNK are the main metabolites detected in the plasma of patients, following a 40min intravenous infusion of 0.5mg/kg racemic ketamine (Zarate et al., 2012, Zhao et al., 2012). Specifically, at the end of a 40min i.v infusion plasma concentrations for ketamine peak at end of the infusion, whereas norketamine peaks at 80min, DHNK at 110min and (2R,6R; 2S,6S)-HNK 230min from the start of the infusion (Zhao et al., 2012).

The ketamine, norketamine and HNK concentration levels that we detected in our study, both at the end of the 40min infusion as well as approximately 2h post the drug administration, seem to be in line with the aforementioned pharmacokinetic model for ketamine's metabolism (Zhao et al., 2012). Indeed, ketamine's levels seems to peak at the end of the infusion whereas they are lower 2h post the drug administration (see Figure 7). Norketamine and both the enantiomers of HNK- (2S,6S)-HNK and (2R;6R)-HNK- show an increase in their concentration 2h post the ketamine administration compared to the end of the infusion (see Figure 7). According to the model however, the peak concentration for HNKs is 230min post the infusion, thus, a later collected sample would be required in order to capture the peak concentrations of these metabolites in the plasma.

Amongst the ketamine metabolites, HNKs have recently gained a lot of interest in depression research since there is evidence, from animal studies, indicating that they could be mediating ketamine's antidepressant actions. Previous pharmacodynamic studies of ketamine and its metabolites have shown that ketamine and norketamine have anaesthetic actions and, at lower doses, increase spontaneous locomotor activity in the rat whereas HNKs do not demonstrate such effects and thus for years they were considered inactive (Singh et al., 2014)

Recent studies, however, have shown that the HNK metabolites have antidepressant actions. When they were isolated and administered in mice, they induced antidepressant behaviours in animal models of depression, such as the FST (Forced Swim Test). Specifically, a positive correlation was detected between the antidepressant response and the plasma levels of (2R,6R)-HNK and the administration of the metabolite alone was enough to induce an antidepressant response. Moreover, in the same study, ketamine's deuteration prevented its conversion to (2S,6S ; 2R,6R)-HNK without changing the total ketamine levels in the brain of mice. In that experiment no antidepressant effects were detected((Zanos et al., 2016) and for review see (Zanos et al., 2018)).

Although some research has produced controversial findings (Shirayama and Hashimoto, 2018). HNKs' antidepressant actions are rather interesting since they challenge the hypothesis that ketamine's antidepressant effects are mainly mediated by NMDAR inhibition. HNKs' potential mechanism of action, involves direct activation of the AMPA receptors (Aleksandrova et al., 2017) and this finding would be in line with other NMDAR antagonists, that do not target the AMPARs and have failed to produce robust antidepressant action (Gould et al., 2019). In our study, we measure HNKs at the end of the infusion as well as 2h post the infusion. The levels of the metabolites (both enantiomes are measured) appear to increase 2h post the drug administration and serve as a useful index of the metabolite levels when the scanning commenced.

The HNK levels in our study did not correlate with the SNK-W or SHAPS scores that capture well-being and anhedonia respectively. This is not surprising since the scores of these questionnaires do not significantly change during the ketamine session, perhaps reflecting the low levels of anhedonia and high levels of well-being in our sample who were not depressed at the time of the study. There is limited information on the time course of ketamine's effects in remitted depressed individuals and so it remains possible that later assessment times (e.g. aligning with the peak of HNK) or at 24hours post-infusion would reveal different effects. It is also possible ketamine does not improve mood and anhedonia *per se* but attenuates deficits in these symptoms. While the neuroimaging reveals specific mechanisms of action on neurocognitive processes (Lally et al., 2014, Lehmann et al., 2016), the translation of these to changes in symptoms is not well specified in the literature and represents a current challenge.

3.2 Memory and Rumination levels in our cohort

3.2.1 Performance on memory scales

In our study, the cognitive performance of our volunteers was assessed using the RAVLT and the Wechsler Memory scale (see Table 3) . Administration of these scales took place before the drug infusion and the time of the administration of those scales was kept consistent between participants and between sessions. This practice was followed for all the assessments that were part of our study in order to ensure that external factors or the experimenter bias do not influence performance in these scales.

The RAVLT could be used to evaluate a wide variety of cognitive functions including short term auditory verbal memory, rate of learning as well as retention of information. The scale could also be used to detect differences between learning and retrieval (Boone et al., 2005). In the literature, the RAVLT has mostly been employed in order to examine mnemonic functions in cases of disease or during aging (Zhu and Paoletti, 2015). The Wechsler memory battery is a neuropsychological set of tests also designed to measure different memory functions. In our study, we did not administer the whole battery but selected the logical memory test to assess the cognitive performance of our volunteers when asked to memorize and subsequently retrieve a short story. The rationale behind choosing those tests was to ensure that the cognitive performance of our participants did not significantly differ between the two study sessions.

Several factors such as fatigue, stress and anxiety can negatively affect cognitive performance ((Alhola and Polo-Kantola, 2007, Scott et al., 2015) for meta-analyses see (Buckman et al., 2018)). Moreover, ketamine has been shown to disrupt memory functions by impeding the encoding of novel information. This deficit is only present when novel information is encoded right after the administration of the drug and it has been shown that ketamine does not influence the retrieval of memories established prior to its administration (Honey et al., 2005). However, these findings suggest that ketamine could, to some extent at least, influence cognitive performance.

In our study, we have used the VAMP, an autobiographical memory fMRI task, to examine ketamine's effects on positive, negative and neutral memory recall. This task does not evaluate memories that are encoded during or right after the drug administration and involves only the retrieval of old and established AMs. The administration of the RAVLT and the logical memory test of the Wechsler Memory scale however, ensure that the cognitive performance of our participants does not differ between session and that when ketamine was administered in the first session that did not influence cognitive function in the placebo session. This way any neuroimaging differences we detect in the VAMP task, between the ketamine and placebo sessions, are due to the effects of ketamine on emotionally valenced memory recall.

We did not expect the performance of our participants on the RAVLT or the logical memory component of the Wechsler Memory Scale to significantly differ between participants and between sessions (see Table 3). For this study we recruited young volunteers and thus we did not expect any serious cognitive impairments related to aging. Moreover, the ketamine administration which could influence the encoding of novel information as required by those scales, when occurred first, was at least 7 days apart from the placebo session. This is enough time for ketamine to be completely metabolised and thus we did not expect to detect a session effect for the performance on these scales.

3.2.2 Rumination

Rumination is one of the most prominent symptoms of depression. The intensity of ruminative thinking could predict the duration of a depressive episode as well as the treatment outcome of individuals with depression (Alhola and Polo-Kantola, 2007, Jones et al., 2008). Moreover, there is evidence indicating that increased rumination persists during remission from depression since high levels of ruminative thinking are also present in previously depressed individuals, even in the absence of other depressive symptoms (Watkins, 2008). In our study, we have used the RRS scale in order to capture rumination and examine any potential relationships between rumination levels and other psychological measures that were acquired during the study as well as between rumination and brain activations during our autobiographical memory fMRI task.

The RRS scale has been developed by Nolen-Hoekseman and her colleagues with the purpose to capture and quantify rumination that is not confounded by symptoms and behaviours associated with depression (Nolen-Hoeksema, 2001). In a large study using this scale in the healthy population, it was shown that there are significant gender differences in the rumination scores captured by RRS with the mean score for healthy males to equal 39.6 whereas healthy females scores around 42 on the scale (Nolen-Hoeksema et al., 1999). In our sample, when the RRS was administered during the screening visit we did not detect any significant gender differences in the scores of our participants (see Table 2). The numerically high scores on the scale, however, indicate that ruination still happens in our group.

When the relationship between rumination (RRS scores) and anhedonia (SHAPS) as well as subjective well-being (SNK-W) were examined no significant correlations were identified between the RRS scores and the baseline scores of those scales on both the placebo as well as the ketamine sessions. The absence of significant correlations between rumination and other behavioural measures in our cohort suggests that there is no direct relation between the rumination levels in our remitted depressed volunteers and elevated anhedonia or reduced subjective well-being.

In the literature, rumination has been associated with the DMN which appears to be activated during self-referential processing as well as AM recall (Grieder et al., 2018, Hamilton et al., 2015). Abnormally increased rumination with a focus on negative AMs, that might be present in depressed and remitted depressed individuals could partly be attributed to an over-active DMN (Default Mode Network) as well as increased activation of the amygdala (Lehmann et al., 2016, Sheline et al., 2009, Young et al., 2016, Young et al., 2018).

In several fMRI studies that have used resting state in order to study connectivity changes in depression it was shown that key regions that form part of the DMN, mainly the PCC and the pACC, present with increased connectivity and that increased levels of DMN activation could be associated with higher levels of maladaptive rumination (Lehmann et al., 2016). Moreover, the PCC along with the amygdala, the hippocampus and temporal areas, present with increased activations during autobiographical memory recall in patients with MDD as well as remitted depressed individuals and those in high risk of developing depression (Gotlib and Joormann, 2010).

The administration of ketamine has been shown to reduce the functional connectivity between the anterior pACC and posterior parts of the DMN in healthy volunteers (Scheidegger et al., 2012). This reduction in the connectivity within the DMN might help depressed patients to become less self-focused and more susceptible to external stimuli thus relieving them from some of the emotional and psychological burden of intense rumination. Although our participants are remitted depressed and thus do not currently experience such intense ruminative thinking and low mood, we did examine whether the scores of the RRS scale would correlate with the brain activations that we observed during the VAMP task. During this task participants are asked to recall positive, negative and neutral AMs. We did not identify any significant correlations between the RRS scores and the brain activations of these areas.

3.3 The effects of ketamine

3.3.1 Acute effects

In addition to its antidepressant action, ketamine is also a psychoactive drug and as such it could alter the subjective qualities of perception, thought and emotion of individuals under its influence, often causing altered interpretations of sensory input. Many of these changes in emotional and sensory perception resemble psychosis symptoms and thus are called psychotomimetic. The psychotomimetic effects of ketamine as well as the acute changes in brain activations that the drug produces are of particular interest especially when ketamine is used as a model of psychosis (Steeds et al., 2015). In research, ketamine has been used in order to validate the glutamate hypothesis of schizophrenia which could explain the negative and cognitive symptoms that are present throughout the illness (Lisek et al., 2017).

The PSI scale has been developed specifically in order to capture and quantify the psychotomimetic effects produced by psychoactive drugs and has been validated not only for ketamine but also for cannabis and other compounds with similar actions (Mason et al., 2008). In our study the scale was administered immediately after the ketamine infusion and a significant increase was observed (Paired-sample t-test, $p<.05$, corrected for multiple comparisons) in all the psychotic-like symptoms that are measured by the PSI. These symptoms include delusory thinking, perceptual distortions, cognitive disorganisation, anhedonia, mania and paranoia (see Figure 9).

In an anaesthesiology study (Bowdle et al., 1998) which aimed to examine ketamine's dissociative effects in relation to the blood concentration levels of the drug in patients recovering from anaesthesia, a linear relationship was identified between ketamine blood concentrations and the psychosis like symptoms that were measured. Moreover, several phMRI studies have investigated associations between changes in subjective ratings of behaviour induced by ketamine and the activation of several brain areas. For example, Deakin et al., (2008) have found that deactivation of the subgenual cingulate and medial PFC correlated with increases in the CADSS – another scale that measures dissociative states (Deakin et al., 2008) while other studies did not identify any correlations between ketamine and the PSI or CADSS ratings (De Simoni et al., 2013). Whether they correlate or not with brain activations, the dissociative effects observed under ketamine are the result of the drug actions on different receptor system including NMDA receptors, sigma and kappa opioid receptors.

Ketamine's binding on the NMDA receptor causes a surge of glutamate release. The excess glutamate and neuronal excitation that follows the NMDA receptor inhibition (for the neuronal/molecular proposed mechanisms of ketamine's action see Figure 1) and subsequent GABA disinhibition could impact areas such as the prefrontal cortical structures, the anterior insula, the thalamus and the amygdala (for review see(Balu, 2016)). These areas play a key role in emotional and self-referential processing and the increased excitation of these areas could account for psychotomimesis (Cohen et al., 2010).

Several of these brain regions are also important for reward processing and appear with altered activations in depression as well as remission (Oldham et al., 2018, Young et al., 2016, Young et al., 2018). However, in our study, the fMRI data were acquired 2h after the ketamine infusion when all the symptoms captured by the PSI have already been resolved and thus we did not expect to identify any significant correlations between the activation of these areas and the dissociative symptoms.

It worth mentioning at this stage that our study has used, as the majority of clinical research involving ketamine in depression, an i.v. infusion protocol with a standard dose of 0.5mg/kg. Contrary to that, most studies in their area of dissociation and psychosis have used a bolus-infusion paradigm. Depression studies that have used a continuous infusion (Berman et al., 2000, Krystal et al., 2013, Zarate et al., 2006) reported that although longer in duration, the infusion is well-tolerated by the majority of participants. However, to our knowledge no study exists that directly compared the tolerability between the two infusion protocols and whether they differ in the amount of psychotomimesis that they would induce. In our study, the drug infusion was generally well tolerated by our participants and in only two instances the infusion was prematurely terminate (5min before the end) due to excessive nausea.

In general, our participants reported very strong dissociative and psychotomimetic effects, which as identified in previous ketamine studies, emerge within 10min from the start of the infusion and subside within 40min of the termination of the drug administration (Zanos et al., 2018). These dissociations, especially in the absence of any previous experiences with psychoactive drugs, were perceived by the majority of our volunteers as unpleasant.

The generally “uncomfortable” state of the volunteers who were experiencing notable dissociative effects in a clinical environment was captured, at least partly, as an increase in the anhedonia subscale of the PSI (see Figure 9), something that in our group we have observed in previous ketamine studies (Wong et al., 2016). Moreover, the reduction in the scores of the SNK-W that was also administered right after the drug administration could also be attributed to the general discomfort that the ketamine infusion could have caused to our volunteers (see Figure 6). The increased anhedonia and reduced subjective well-being identified after ketamine’s administration would take time to normalise and that might delay the detection a significant improvement of anhedonia produced by ketamine as part of its antidepressant action. These findings are further discussed in the section that follows.

3.4 Anhedonia and Subjective Well Being

In our study anhedonia was not only measured right after the drug administration by the PSI subscale, but the SHAPS as well as the SWN-K were also used to asses anhedonia and subjective well-being, respectively. These scales were administered at the start of the day, right after the drug infusion as well as 2h after the end of the drug administration.

In our study however, we did not observe a reduction in the SHAPS scores after ketamine administration (see Figure 5). In the literature, higher SHAPS scores have been associated with poorer treatment outcome in MDD and the scale is also sensitive to the "early" antidepressant effects of ketamine (Argyropoulos and Nutt, 2013). Specifically, studies that have used the SHAPS to explore ketamine's effects in anhedonia during depression reported a significant reduction in the SHAPS scores as early as 40min post ketamine administration, indicating ketamine's positive and rapid effects on anhedonia (Lally et al., 2014). A possible explanation for the absence of any changes in anhedonia 2h after ketamine, when the antidepressant effects of the drug should be detectable, could be attributed to the fact that for this study we chose to recruit remitted depressed volunteers.

Our study participants do not experience any depressive symptoms at the time of the study and although anhedonia could persist during remission, in our cohort the baseline SHAPS scores are very low (see Figure 5). The low baseline scores indicate either the absence of anhedonia or its presence in very low levels. If anhedonia is indeed absent from our cohort then ketamine is not expected to produce any effect. If however, anhedonia is present but in very low levels then it is possible that ketamine, might require more time to produce any significant improvements in the hedonic capacity of our participants. It is moreover possible that the SHAPS might not have the sensitivity required to capture any very subtle effects that ketamine might have on the very low anhedonia levels of our cohort.

The stressful and unpleasant effects that acute ketamine administration induces and have been experienced by the majority of our participants (see Figure 9) could offer an alternative explanation for the absence of any significant improvement in anhedonia 2h post the ketamine administration. Research has shown that anhedonia and stress are integrally related (Pizzagalli, 2014, Stanton et al., 2019, Treadway and Zald, 2011). According to Meehl's theory, the ability to experience pleasure and anticipate it serves to protect us against the negative influences of stress and the potential development of psychiatric disorders including depression. Consistent with that theory animal models have shown that

chronic mild stress decreases the sensitivity to reward and produces anhedonic behaviour(Moreau, 2002) but also that dysfunction in the reward system could be reversed by antidepressants as well as ketamine (Belujon et al., 2014).

The dissociative effects that are experienced by the majority of our participants under ketamine could be perceived as potentially stressful, causing an increase in anhedonia. This significant increase in anhedonia during the ketamine administration compared to placebo, has been captured by the anhedonia subscale of the PSI (see Figure 9). Moreover, the SNK-W scale which was administered right after the infusion, showed lower scores of well-being after ketamine administration (see Figure 6). This finding was not present during the placebo session, although the interaction was not statistically significant. It is thus possible that 2h after the ketamine infusion, anhedonia which is reduced due to the unpleasantness of the infusion would trend towards normalization. This could be indirectly reflected in the SHAPS score which 2h after the ketamine infusion, equals that of the baseline (start of the day). No correlations were identified however, between the PSI scores- total scores and subscale scores- with the SHAPS scores after ketamine.

An interesting observation in our study concerns the SHAPS scores of the placebo session. A significant reduction in the SHAPS scores was observed between the baseline scores and those collected 2h post the saline infusion indicating a decrease in anhedonia. This decrease in anhedonia, 2h post the placebo administration, could be attributed to the participants' reduced anxiety on the placebo day and/or the unavoidable stress of an intravenous infusion which is rather invasive. A common problem with ketamine studies is the absence of a good placebo. In our study and during the ketamine infusion the side effects produced by the drug, made it practically impossible to maintain the blind of the study since both the participants and the researches could accurately guess when ketamine was administered. Anhedonia measured after the infusion, could thus be decreased independent of whether the placebo session precipitates or follows the ketamine day, since in both cases the stress of the around the infusion and/or ketamine's side effects is lifted.

In addition to the SHAPS, the SNK-W was used in order to assess the subjective well-being of our participants at the start of each study day, right after the infusion and 2h post the drug administration (see Figure 6). The SWN-K is a well validated scale that includes questions around mental functioning, social integration, emotional regulation, physical functioning and self-control and assesses the "in the moment" well-being of participants. The scale has previously been used in order to assess subjective well-being, mainly in psychosis (Maurino et al., 2012). This scale was designed with the specific aim of measuring patients' subjective experiences regardless of their mental state and medication stabilization. The SWN-K, to our knowledge has never been used before in a depression study. However, it has been extensively used in psychosis studies to assess participants' well-being in relation to their depressive symptoms and treatment outcome (Haring et al., 2013). Moreover, due to the nature of the questionnaire that assess the current mood of individuals and without asking them to imagine how they would feel in different scenarios, we believed it would be more sensitive to capture mood changes on the study day.

Our results show that right after the drug infusion the SNK-W scores decrease, however, they return to baseline 2h after the ketamine administration. During the placebo session there are no significant changes in the scores of this scale. We believe that the lower SNK-W scores right after the ketamine infusion could further reflect the stressful experience of a psychoactive drug infusion. This effect, as we mentioned before, might also be reflected in the increased anhedonia measured by the PSI right after the ketamine infusion.

4 Conclusions

Analysis of the behavioural data that were obtained from this study helped us better comprehend the cognitive characteristics of our volunteers and examine the acute and delayed- 2h post infusion- effects of ketamine.

Rumination was present in our cohort of remitted depressed volunteers, however the luck of studies around remitted depressed individuals with the use of the RRS scale and the fact that the scores in our study did not correlate with brain activations during AM recall, make it very difficult to link rumination in our cohort with depression-related processes. Moreover, in our cohort of remitted depressed volunteers we did not identify significant levels of anhedonia (SHAPS) or low mood (SNK-W) at baseline. The absence of anhedonia and the presence of rumination, taken together, constitute a rather interesting finding. Previous research has shown that anhedonia and rumination are two cardinal symptoms of depression that tend to persist, to a lesser extent, during remission (Pizzagalli, 2014, Abela and Hankin, 2011). The relationship between these symptoms and the neuronal pathways that could mediate reward processing as well as ruminative thinking is still unclear, especially in the absence of clinically significant depressive symptoms. However, it is possible that the altered neuronal processes that underlie those symptoms might still be present but not behaviourally declared in a detectable way. The absence of anhedonia in our group, for example, does not necessarily mean that altered neuronal processing in brain areas associated with reward is also absent.

As far as the drug effects are concerned, the administration of the PSI, right after the drug infusion, allowed us to capture the dissociative effects that ketamine produces during its administration as well as some of the discomforting and unpleasant aspects of an i.v. infusion of a psychoactive drug. The anhedonia and subjective well-being of our participants were measured at the start of each study session as well as 2h post the drug administration. The data from these scales indicate that 2h after its administration, when the antidepressant effects of ketamine are detectable, there are no significant changes in the anhedonia and subjective well-being in our cohort. This finding is not very surprising since our remitted depressed volunteers do not report high levels of anhedonia at the start of the day. Consequently, ketamine is not expected to produce a marked improvement in that symptom. Moreover, the drug administration might cause some discomfort to our participants and thus the two-hour interval between the infusion and the last administration of the SHAPS might not be enough to capture ketamine's antidepressant action.

Take together all these findings highlight the suitability of our cohort for the study of the potential mechanisms of the early antidepressant effects of ketamine. Remitted depressed individuals, as the ones recruited for our study, seem to more closely resemble depressed patients both behaviourally but perhaps also in a brain activations level. In the two chapters that follow, we will try to investigate how AM recall and rumination as well we reward anticipation might be altered by ketamine, 2h after its administration, in our cohort. We will try to discuss the fMRI finding in relation to the behavioural data presented in this chapter and link them to previous research findings. We hope that this would help us understand how ketamine might exert its early antidepressant effects.

The MID task

1 Introduction

In this chapter we will examine the delayed effects of ketamine on brain areas that are important for reward processing. The MID task is a well validated task that has been specifically developed in order to study reward processing in the MRI scanner (Knutson et al., 2001). In our study the task was performed 2h after the ketamine and placebo administration. The reward anticipation and the feedback phases of the task were analysed in order to examine ketamine's delayed effects in brains activations in our cohort of remitted depressed volunteers.

In order to better understand how ketamine might influence brain activations during the MID task, we need to have a good understanding of the brain areas that are important for each of the different components of this task. For that purpose, first we isolated the placebo group and conducted a whole brain analysis in order to identify those brain regions that are significantly activated during reward anticipation and feedback, compared to the neutral component of the task. We also examined reward magnitude to identify those brain areas that might be implicated in the anticipation and feedback of a high and lower in value monetary rewards. Finally, we looked at "no win" trials, focusing only on the feedback phase, and aimed to identify brain areas that are important for "no win" feedback, compared to neutral feedback. We also looked at how the magnitude of reward during these unsuccessful "no win" trials might alter brain activity.

An ROI analysis was conducted in the placebo group focusing on the role of the striatum, the amygdala and the insula for reward processing during the MID. These three brain areas are very important for reward processing and have also been identified in the literature as areas that present with altered activations in depressed and remitted depressed individuals. We examined the role of these ROIs in the different contrasts that were created for the MID task.

The effects that ketamine administration might have in brain areas that are important for the MID was investigated by comparing the ketamine and placebo sessions for all the contrasts of interest. A whole brain level analysis was conducted to investigate ketamine's antidepressant action during reward anticipation and feedback. Ketamine's antidepressant effects during reward, different magnitudes of reward as well as during feedback for "no win" trials were also examined. Finally, we compared the ROI activations between ketamine and placebo in order to examine whether and how ketamine, 2h after its administration, would influence the activation of brain areas with a known role during reward processing. For this analysis the ROI activations were examined under different task conditions and compared between the two study sessions (ketamine session vs placebo session).

The behavioural data of the MID task, including the RT (Reaction Time), the total winnings as well as the number of "win" and "no win" trials were also analysed for the placebo and ketamine sessions. Poor performance to the task, either during the ketamine or the placebo session, could exclude participants from the data analysis as we wanted to ensure that only trials from sufficiently engaged participants would be included. The behavioural data obtained from the two drug sessions were compared to examine whether ketamine would significantly alter performance to the MID task.

Note

For the analysis and discussion of the MID task we will be referring to the MID trials as successful trials when the expected reward was actually earned by the participants. The unsuccessful trials are those trials where the signalled reward was not obtained and are different to the neutral trials where the cue indicates that this trial is not be associated with a reward. Failure to obtain the signalled reward is not referred to as "loss" since in our version of the MID, unlike other versions with an actual loss condition, the amount of money not won is not subtracted from the participants winnings but just not added to the total.

2 Results

Performance

The MID task is a reaction time task and successful performance of this task requires participants to make fast responses in order to win monetary rewards. The magnitude of the reward is signalled by the presentation of a specific cue. In order to perform the task successfully, participants are required to pay sufficient attention to the cues that precipitate the presentation of the target (circle with one line, circle with two line, triangle) and respond quickly to the target itself (white square).

In order to ensure that our participants were appropriately engaged to the task, both during the ketamine as well as the placebo session, we looked at individual task performance during the two drug sessions and removed from our analysis any subject that exceeded 2SDs of the session average either in percentage of hits on win trials or on reaction time. To further ensure participants attention to the task, we excluded any individual with a response rate less than 66%. Response rate refers to a successful button press within the entire 500ms response window, regardless of whether the trial is a “win” or a “no win” trial.

Although these cut offs are relatively conservative, we believe that they ensure our volunteers’ understanding and sufficient engagement to the MID task. Thus, any effects we might observe when we look at the placebo data or compare the ketamine with the placebo sessions can be attributed to the task and the effects of the drug and not due to fatigue or failure to successfully perform the task.

Based on these criteria, only one participant was excluded from this analysis.

2.1.1 Response rate and total MID winnings

Across all the trials of the MID task, including neutral trials where no reward is expected, participants maintained sufficient engagement to the task. Overall, our volunteers responded to at least 90% of the MID trials that required a button press, namely low win, high win and neutral trials.

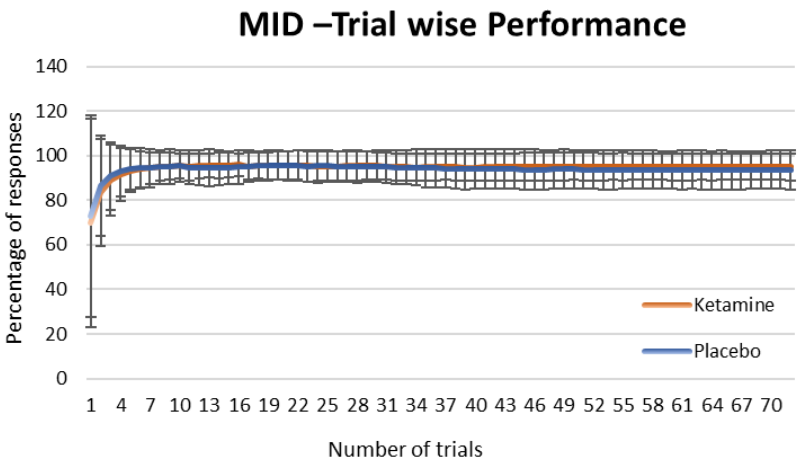

Figure 10. *Cumulative response rate (with SD bars) over duration of the task on trials that required a response (neutral trials, low win trials and high win trials). Ketamine did not significantly alter (paired t-test, p>.05) the trial wise response performance.*

Comparison between the ketamine and placebo sessions did not reveal a significant effect of the drug, 2h post administration, in the overall response rate.

In terms of the total amount of money that participants won during the ketamine and placebo session, no significant changes were identified between the two drug conditions. No significant changes were also identified when the reactions times for low, high and neutral reward were examined and compared between ketamine and placebo. Although in both the ketamine and the placebo sessions, reaction time significantly decreased when it was compared between the different reward magnitude trials.

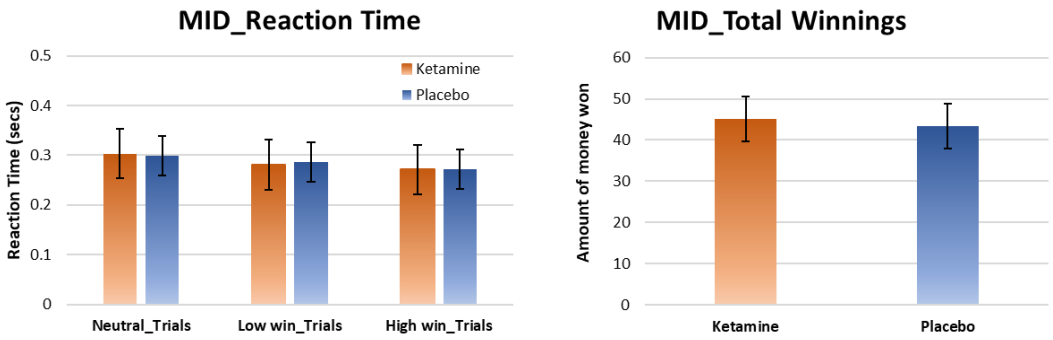

Figure 11. *The mean reaction time and the total amount of money won per trial did not significantly change between the ketamine and placebo sessions (paired t-test, p> .05). A significant reduction was identified in the reaction times of high, low and neutral win trials for both ketamine(Repeated Measure Anova, F(2, 36)=23.17, P<.001) and placebo (Repeated Measure Anova, F(2, 36)=34.27, P<.001)*

2.2 Neuroimaging Results

2.2.1 Reward anticipation in the placebo group at a whole brain level

2.2.1.1 Anticipation of all win vs neutral

` The first aim of this analysis was to replicate previous research where the MID task was engaging brain areas that have previously been identified as important for the task related processes of reward anticipation and feedback. As a result, for this initial analysis of the task, we focused on the reward anticipation phase of both high and low win trials and compared it to reward anticipation of neutral trials where no reward is expected.

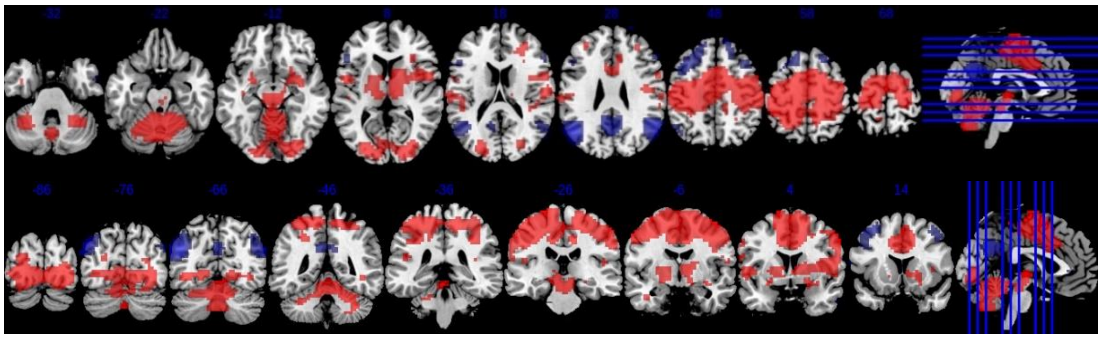

Figure 12. *A whole brain analysis revealed that several brain regions presented with significant increased (red) and decreased (blue) activations in the placebo group. The contrast that was examined here combined the anticipation phase of high and low win trials and compared to anticipation phase of the neutral trials, where no*

When all the win trials (high win trials and low win trials combined) of the task were compared to the neutral trials of the MID, during which no reward is expected, very strong increases and decreases were found in brain areas that have previously been identified as important for reward anticipation and are implicated in this task. Specifically, significant clusters of increased brain activations were identified in the SMA (Supplementary Motor Area), precentral regions, occipital areas, the cerebellum and the thalamus whereas decreases were identified in clusters including the middle temporal gyrus, the angular gyrus and the superior frontal gyrus (Figure 12). The significant clusters of increased and decreased brain activity are summarised in Tables 6 and 7 respectively. While the ventral striatum is included as part of the clusters, but we examined this *a priori* region using ROI analysis (see below).

Cluster level pFWE_corr	Peak level pFWE_corr	Brain Region
0.000	0.000 0.000	SMA (0 -4 59) Precentral_L (-38 -15 59)
0.000	0.001	Occ_Mid_L (-22 -86 0)
0.001	0.001	Cerrebelum_L (-4 -45 -13)
0.000	0.003 0.004	Lingual_R (22 -86 -10) Calcarine _L (15 -86 0)
0.019	0.025	Supramarginal_R (60 -22 40)
0.031	0.030	Thalamus (11 -15 10)
0.031	0.043	Precentral_R (38 -11 59)

Table 6.*Several significant clusters present with significantly increased activations when the anticipation phase of high reward and low reward win trials is compared to the neutral trials.*

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Cluster level pFWE_corr	Peak level pFWE_corr	Brain Region
0.000	0.000 0.000	Temp_Mid_L(-49 -64 23) Angular_L (45 -68 33)
0.001	0.000	Angular_R (60 -56 26)
0.002	0.000	Precuneus_L (0 -60 33)
0.001	0.001 0.009	Front_Mid_L (-34 19 56) Front_Sup_L (-19 34 53)
0.017	0.004	Front_Sup_R (19 34 56)

Table 7. *The clusters that present with significantly decreased activations when the anticipation phase of high reward and low reward win trials is compared to the neutral trials.*

2.2.1.2 *Reward magnitude during reward anticipation*

In order to examine the role of reward magnitude during the anticipation phase of the task we contrasted the anticipation phase of high win trials to the anticipation phase of low win trials. The brain areas that present with increased activations when a high reward was anticipated compared to a low win trial included the cingulum, the SMA and inferior occipital areas (Figure 13). Table 8 summarises the voxel clusters that presented with increased activation during high compared to low reward anticipation. The ventral striatum is included as part of the cluster but examined this *a priori* region using ROI analysis (see below).

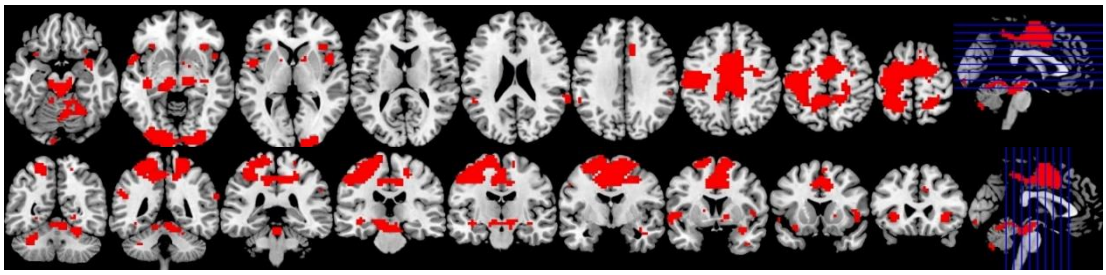

Figure 13. *When the anticipation phase of low reward and high reward trials were compared, significant increases (red) were identified in frontal and occipital brain areas.*

Cluster level pFWE_corr	Peak level pFWE_corr	Brain Region
0.000	0.000 0.001 0.005	Cingulum_Mid_R (8 8 46) Front_Sup_L (-19 -4 69) SMA_L (0 -8 56)
0.000	0.008 0.027	Occipital_Inf_R (26 -98 0) Occipital_Inf_L (-11 -98 -7)

Table 8. *The clusters that presented with increased activation, when reward magnitude was examined, are presented here along with the brain areas (MIN coordinates) where the peak of the cluster is located.*

2.2.2 Feedback in the placebo group at a whole brain level

2.2.2.1 Feedback from successful high reward and low reward MID trials, compared to the feedback of neutral trials

The feedback phase of the successful trials of the MID task (high and low win trials) was analysed in the placebo group in order to identify brain areas that are important for this phase of the task. We found that earning the signalled reward, despite its magnitude, produced significant decreases in brain areas that included the precentral gyrus, superior frontal regions, inferior parietal and middle frontal areas (see Figure 14). Significant increases were not identified for this component of the task.

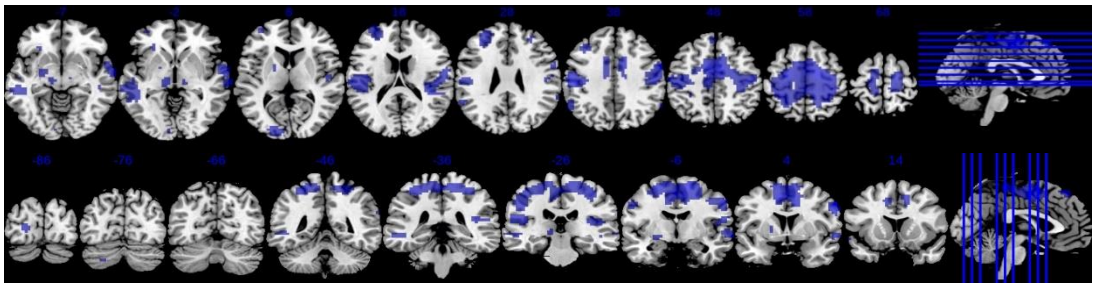

***Figure 14**. Significant decreases (blue) in the activation of frontal brain areas during the feedback phase of successful high reward and low reward trials when compared to the feedback component of neutral trials.*

Cluster level pFWE_corr	Peak level pFWE_corr	Brain Region
0.000	0.007 0.009 0.016	**Precentral_R (60 4 36)** **Front_Sup_R (19 -11 63)** **Front_Inf_R (49 -22 40)**

***Table 9**. The clusters that presented with decreased activation during the feedback phase of the successful MID trial are presented in Table 4 above. The MNI coordinates of the peak of the cluster is also included.*

2.2.2.2 *Feedback from unsuccessful high reward and low reward MID trials, compared to the feedback of neutral trials*

When the feedback phases of low and high win unsuccessful trials, where participants did not win the expected reward, was contrasted to the neutral phase of the task, significant increases were identified in occipital, parietal and temporal brain areas. The post central region presented with decreased activations during the feedback phase of unsuccessful trials compared to the neutral ones (see Figure 15 and Table 8).

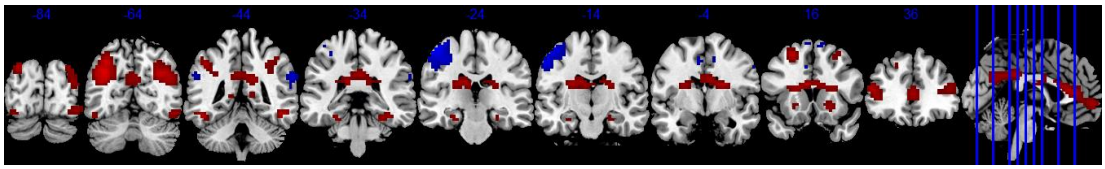

Figure 15. *Occipital, parietal and temporal brain areas presented with significantly increased activations during feedback of unsuccessful trials compared to feedback from neutral MID trials. Significantly decreased activations were identified in the post central region for the same contrast.*

Cluster level pFWE_corr	Peak level pFWE_corr	Brain Region
0.000	0.000 0.002	Occipital_Mid_L(-30 -71 36) Parietal_Inf_L (-34 -60 46)
0.016	0.003	Temporal_Inf_L (-49 -52 -13)
0.000	0.010 0.018	Occipital_Mid_R (34 -64 33) Frontal_Inf_Tri_L (-45 34 13)
0.030	0.016	Frontal_Sup_L (-22 19 53)
0.002	0.019	Temporal_Inf_R (52 -52 -13) Fusiform_R (26 -30 -20)
0.004	0.012	Post_Central_L (-49 -19 56)

Table 10. *The clusters that presented with significantly increased and decreased activations during the feedback phase of the unsuccessful MID trials are presented in this table. The MNI coordinates of the peak of the cluster are also included.*

2.2.2.3 *Reward magnitude during the feedback phase of the MID task*

When the feedback phase of high win trials was contrasted to the feedback phase of low win trials we found that one cluster located in the cingulum presented with significant decreases for the high win feedback phase compared to the low win feedback phase of the MID task (see Figure 16 and Table 10).

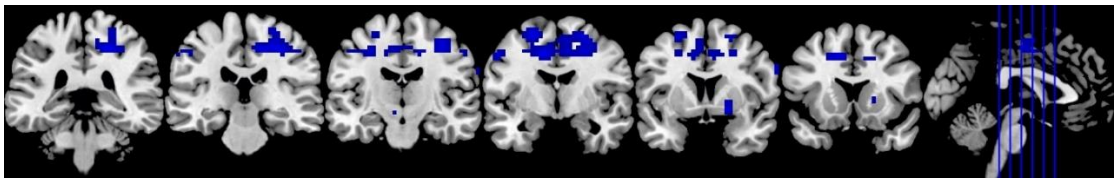

Figure 16. *The cingulum presented with significantly decreased activation when the feedback phase of high reward MID trials was compared to the feedback phase of a low win trials.*

Cluster level pFWE_corr	Peak level pFWE_corr	Brain Region
0.000	0.034	Cingulum_Mid_L(19 -30 49)

Table 11. *One cluster of significant decreases in brain activity was identified when reward magnitude was examined for the feedback phase of MID trials. The MNI coordinates of the peak correspond to the left middle cingulate cortex.*

2.2.3 The effects of ketamine on the MID task

2.2.3.1 Ketamine and reward anticipation

Ketamine did not produce any significant changes during reward anticipation when high win and low win trials were combined and compared to the neutral trials at a whole brain level. Moreover, no significant changes were identified when the level of reward magnitude (high win anticipation vs low win anticipation) was compared between the ketamine and placebo sessions.

2.2.3.2 Ketamine and feedback

When the feedback phase of the task, for both successful and unsuccessful MID trials, was examined and compared between the ketamine and placebo sessions, we did not identify any significant changes on brain activations. Moreover, no significant changes were identified when the feedback phase of high win trials was contrasted to the feedback phase of the low win trials.

2.2.4 ROI Analysis

2.2.4.1 In the placebo group

The role of the *a priori* identified ROIs was examined during the MID task. The striatum, the amygdala and the insula presented with significant activations during reward anticipation and feedback of the high and low win reward trials compared to the neutral trials of the MID. Moreover, several of these ROIs presented with significant activations when the role of reward magnitude during anticipation and feedback was examined.

Since we did not have a strong hypothesis as to how the different components of the MID task –anticipation, feedback and reward magnitude- might alter the role of our ROIs concerning their lateralisation, we decided to primarily investigate our ROIs bilaterally. However, we did examine the involvement of our ROIs by extracting beta values for the left and right ROI as well. This analysis is exploratory, thus we did not apply multiple comparison correction to the significant p values obtained from it. The results from this analysis can be found in Appendix G. Table 11 showa only the bilateral ROIs and highlights in red the mean beta value and standard deviation of thosethat survived Bonferroni correction for multiple comparisons.

		Bilateral				
		Caudate	Insula	Putamen	VS	VTA
Anticipation	High and Low_win_vs_Neutral	Mean = -0.39, SD = ± 0.61	Mean = -0.28, SD = ± 0.37	Mean = -0.24, SD = ± 0.34	Mean = -0.31 SD = ± 0.45	Mean= -0.29 SD = ± 0.39
	High_win_vs_Neutral	Mean = 0.30, SD = ± 0.66	Mean = 0.26, SD = ± 0.41	Mean = 0.27, SD = ± 0.41	Mean = 0.34, SD = ± 0.48	Mean = 0.46, SD = ± 0.41
	Low_win_vs_Neutral	Mean = -0.39, SD = ± 0.61		Mean = -0.39, SD = ± 0.61	Mean = -0.39, SD = ± 0.61	
	High_win_vs _Low_win			Mean = -0.33, SD = ± 0.77	Mean = -0.41, SD = ± 0.71	Mean = -0.29, SD = ± 0.76
Feedback	High_and _low_win_vs_Neutral	Mean = -0.18, SD = ± 0.39			Mean = -0.15, SD = ± 0.34	
	High_win_vs_Neutral			Mean = -0.55, SD = ± 1.14	Mean = -0.35, SD = ± 0.93	
	Low_win_vs_Neutral					
	High_win_vs_Low_win	Mean = -0.46, SD = ± 0.92	Mean = -0.30, SD = ± 0.64	Mean = -0.39, SD = ± 0.61	Mean = -0.48, SD = ± 0.70	Mean = -0.59, SD = ± 0.65
	High_win_loss_vs_Neutral	Mean = -0.83, SD = ± 0.99	Mean = -0.42, SD = ± 0.92	Mean = -0.99, SD = ± 0.86	Mean = -0.84, SD = ± 1.02	
	Low_win_loss_vs_Neutral		Mean = 0.15, SD = ± 0.52	Mean = 0.22, SD = ± 0.45	Mean = 0.14, SD = ± 0.36	Mean = 0.34 SD = ± 0.37
	All_win_vs_All_loss	Mean = -0.39, SD = ± 0.61	Mean = -0.39, SD = ± 0.61	Mean = -0.39, SD = ± 0.61	Mean = -0.39, SD = ± 0.61	Mean = -0.39, SD = ± 0.61

Table 12.The mean activation values are shown here for each bilateral ROI along with the SD. The values in red are the ones that have survived the Bonferroni correction for multiple comparisons

2.2.4.2 *The effect of ketamine*

The role of our a priori defined ROIs was examined between the ketamine and placebo sessions. We investigated the role of our ROIs for all the task contrasts that were explored under placebo namely, the reward anticipation, magnitude of reward and the feedback components of the MID trials. In this section we will present the task contrasts for which significant results were obtained. ROI analysis involved, as in the placebo session, bilateral ROIs and the corresponding left and right lateralized brain areas. Bonferroni correction was applied to the results of this analysis and only ROIs that survived this correction are presented.

2.2.4.3 *Reward Anticipation*

We found that during the anticipation phase of low reward trials compared to neutral trials, when no reward is expected, ketamine significantly increased the activation of the caudate bilaterally. Significant activations were also identified for the left caudate (not corrected for multiple comparisons). In this analysis the anticipation phase of the high win trials included both successful as well as unsuccessful MID trials.

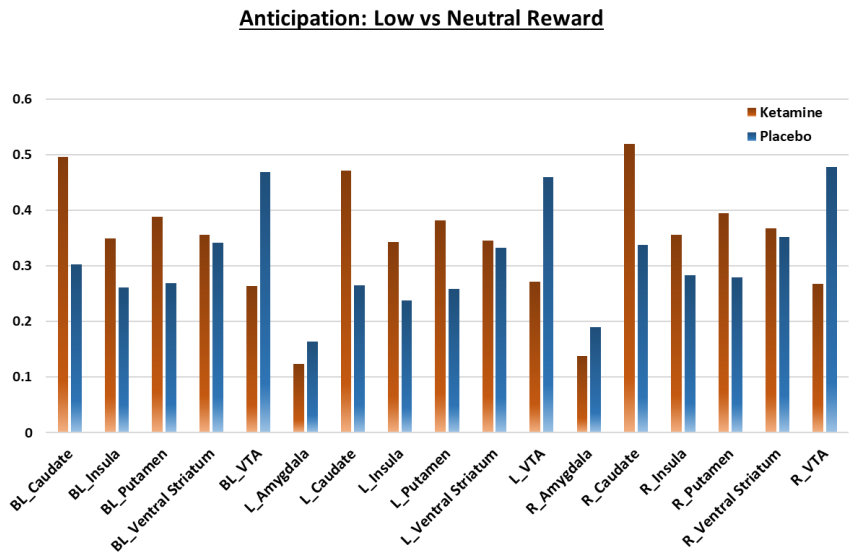

Figure 17. *Ketamine, during the anticipation of a low reward compared to neutral, significantly increased the activation of the bilateral and left caudate. The red asterisk indicates that the change was still significant after Bonferroni correction.*

2.2.4.4 *Feedback of a successfully obtained reward*

During the feedback phase of the MID we found that ketamine significantly increased the activations of the bilateral insula and the ventral striatum during the feedback phase of a low win trials compared to neutral. For this analysis only successful trials were included.

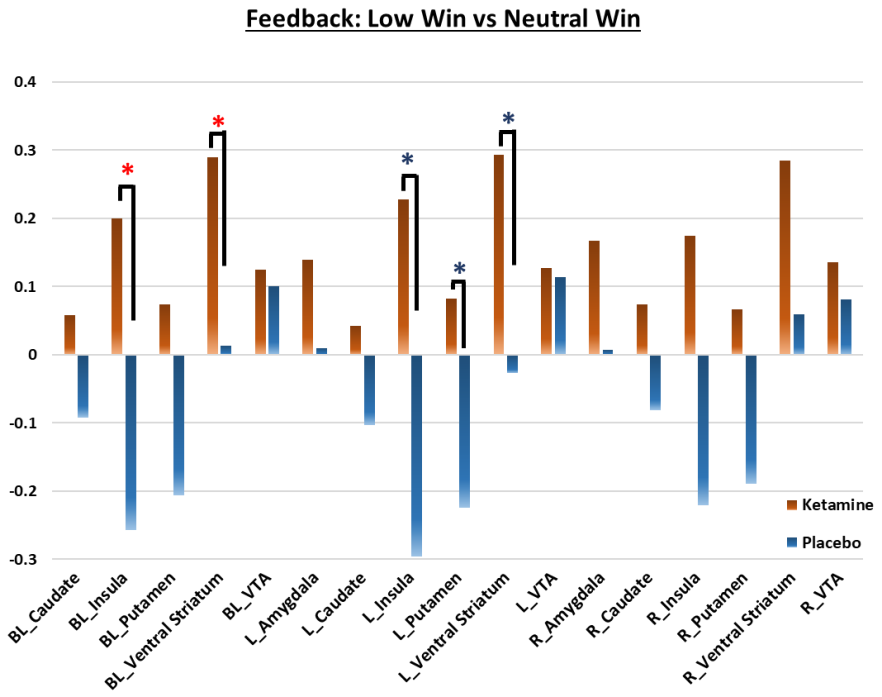

Figure 18. *Ketamine, 2h post the drug administration, significantly modulated the activation of the ventral striatum and the insula during the feedback phase of a successfully obtained reward. The red asterisk indicates that the change was still significant after Bonferroni correction. In our exploratory analysis, the left ventral striatum as well as the left insula and the left putamen were also significantly modulated by ketamine for that contrast.*

2.2.4.5 Feedback of unsuccessful MID trials

2.2.4.5.1 High Reward loss

When the feedback phase for a high reward unsuccessful trial was compared to the feedback phase of neutral trials, it was found that ketamine significantly increased the activation of the bilateral insula.

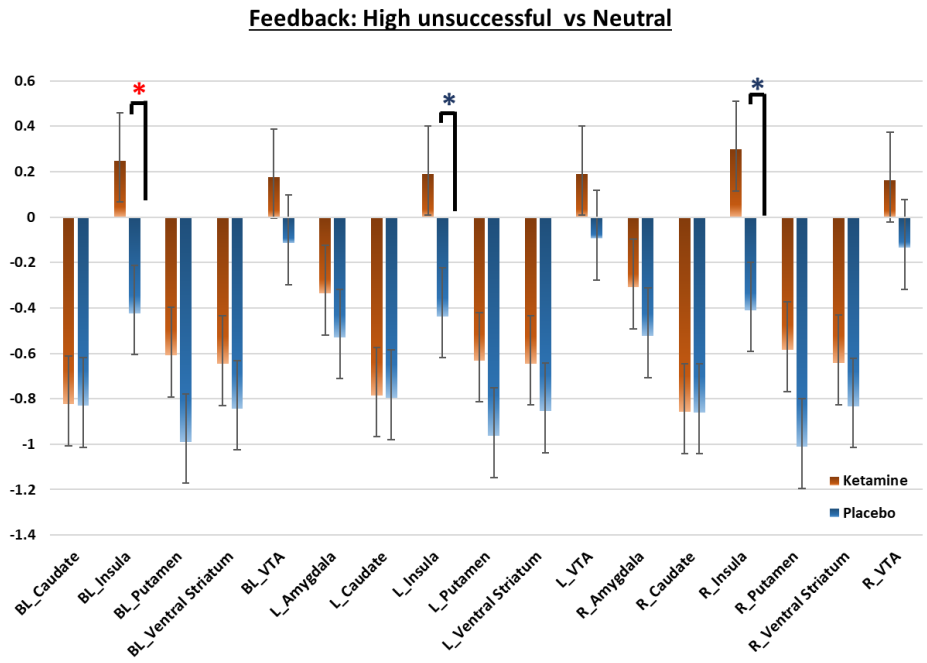

***Figure 19**. During the feedback phase of MID trials where a high reward was anticipated, the bilateral insula, presented with increased involvement after ketamine compared to the placebo session. The red asterisk indicates that the change was still significant after Bonferroni correction. The left and right insula were also significantly modulated by the drug.*

2.2.4.5.1.1 Low Reward unsuccessful trials

The feedback phase of a low reward unsuccessful trials was compared to the feedback phase of neutral trials and it was found that ketamine significantly increased the activation of the caudate, bilaterally.

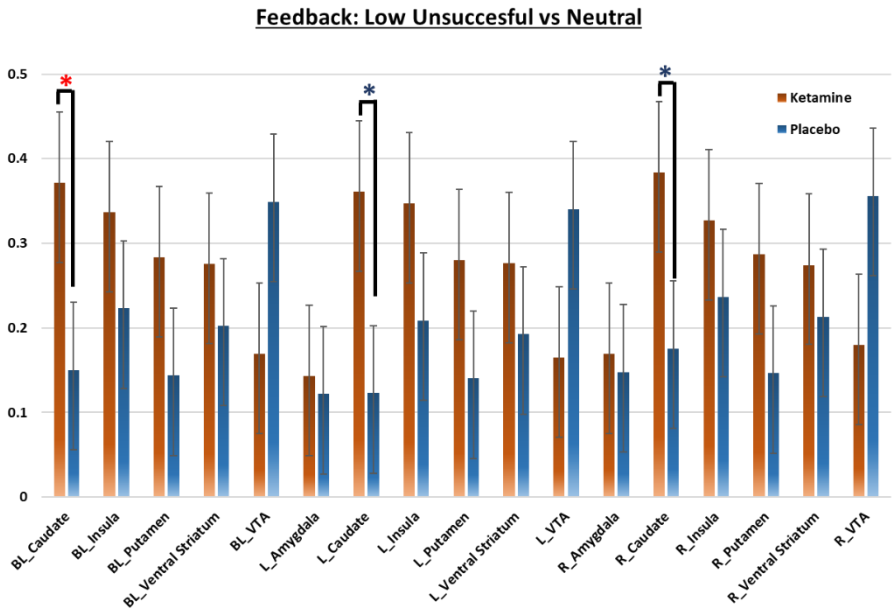

Figure 20. *The bilateral caudate appears to be significantly more activated after ketamine compared to placebo when feedback for unsuccessful low reward MID trials is presented to participants. The red asterisk indicates that the change was still significant after Bonferroni correction. The left and right caudate also present with increased activations for that contrast.*

2.2.4.5.1.2 High and Low Reward Loss

When the feedback phase of both high and low reward unsuccessful trials was compared and contrasted to feedback from neutral trials, ketamine significantly altered the activation of the bilateral VTA, left amygdala and left VS.

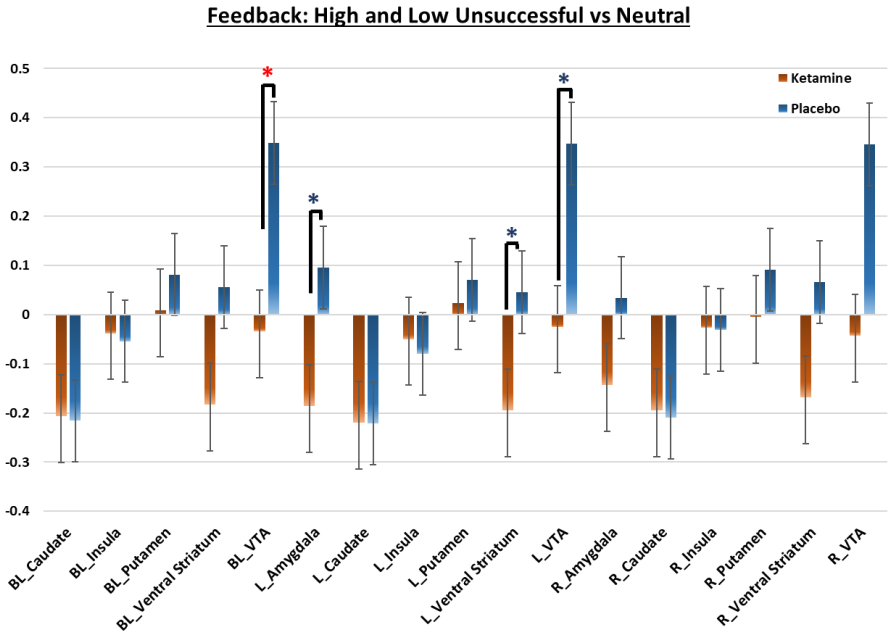

Figure 21. *When the feedback phase for high and low reward unsuccessful trials was examined between the ketamine and placebo sessions, we found that there increased involvement for the bilateral VTA and the left amygdala for that contrast. The red asterisk indicates that the change was still significant after Bonferroni correction. The left ventral striatum and left VTA also present with increased involvement for that contrast.*

2.2.4.6 Effect of session

In order to investigate whether there is an effect of session in our study, we analysed separately the MID data that were acquired when ketamine was administered on the 1st session. These data were compared to the ones acquired when placebo was administered on the 1st session. For this analysis a total of 18 participants were included in each group. Reward anticipation as well as feedback and the role of reward magnitude were examined and compared between the two groups using a two-sample t-test.

The results from this analysis did not reveal any effect of session on the MID task.

3 Discussion

Introduction

The MID task has been used in this study in order to examine the delayed effects of ketamine on motivational anhedonia in remitted depressed volunteers. Specifically, we investigated whether ketamine, 2hours after its administration, when the antidepressant effects of the drug become detectable, would alter the performance and brain activations produced by this task, compared to placebo. For that purpose, the anticipation and feedback phases of the MID trials were examined in the placebo group and compared between the ketamine and placebo sessions. Moreover, the effect of ketamine on reward magnitude during anticipation and feedback was also investigated. An ROI analysis was conducted in order to detect any delayed effects of ketamine on *a priori* defined brain areas that are important for reward processing.

Summary of findings in the placebo group

Analysis of the MID task in the placebo group revealed that anticipation of an expected reward, despite its magnitude, produced significant increases in the activation of precentral brain areas, the cerebellum and the thalamus (see Figure 12). Decreased activations were identified, for the same contrast, in temporal areas, the precuneus and middle and superior frontal regions. When the effects of reward magnitude were examined, it was shown that during the anticipation of a high reward, compared to a low reward, brain activity significantly increased in the anterior cingulum, superior frontal regions including the SMA and inferior occipital areas (see Figure 13).

During the feedback phase of the task, decreased activations were found in inferior and superior frontal areas when the monetary reward, despite its magnitude, was successfully won by the participants (see Figure 14). When the feedback phase of unsuccessful trials- where no reward was obtained - was compared to that of neutral trials, it was shown that significant increases were produced in several brain areas including frontal superior regions, occipital areas and the fusiform gyrus. Significant decreases for that contrast were located in the left post central gyrus (see Figure 15). We also found that during the feedback phase of successful high win trials, the activation of the middle cingulum significantly decreased, compared to the feedback phase of low win trials(see Figure16).

When the role of the striatal ROIs was examined, it was shown that during reward anticipation the activation of the insula, the putamen and the VS bilaterally, decreased significantly during reward anticipation of "win" compared to neutral trials. Moreover, the VS and VTA presented with significantly decreased activation when anticipation of high win trials was compared to low win trials. During the feedback phase of the MID the VTA presented with decreased activity for unsuccessful low win trials compared to neutral trials (see Table 11).

Summary of findings for ketamine session vs placebo session

Ketamine, 2hours after its administration did not significantly alter the performance to the MID task (see Figure 11). Specifically, when the total amount of money that was won during the task and the average reaction times for low win, high win and neutral trials were compared between the ketamine session and placebo session, we did not identify any significant changes between the drug conditions. Moreover, participants maintained sufficient engagement to the task since they responded to at least 90% of the trials both after placebo and ketamine administration (see Figure 10).

At a whole brain level, ketamine did not produce any significant changes in any of the contrasts that were examined, for the anticipation and feedback phase of the task. Ketamine also failed to produce any significant effect when reward magnitude was examined in the whole brain analyses.

When the effects of ketamine on the a priori defined ROIs were examined, we found that during reward anticipation, ketamine significantly increased the involvement of the bilateral caudate when a low reward was anticipated compared to neutral trials (see Figure 17). The drug effects were more prominent during the feedback phase of the MID task. Specifically, when the feedback phase of successful low win trials was compared to neutral, ketamine increased the activation of the insula and the VS, bilaterally (see Figure 18). When the feedback phase of unsuccessful trials was examined, it was found that ketamine significantly increased the activation of the VTA, bilaterally. Moreover, during the feedback phase of unsuccessful high win trials, compared to neutral trials, ketamine significantly increased the activation of the bilateral insula (see Figure 19) whereas during the feedback phase of unsuccessful low win trials compared to the feedback phase of neutral trials, ketamine significantly increased the activation of the caudate, bilaterally (see Figure 20).

3.1 The MID task in the placebo group

3.1.1 Whole Brain Analysis

The MID task is a motivation task, specifically designed to examine reward processing with the use of fMRI. The design of the task allowed us to distinguish between the anticipation and feedback phases of each MID trial and identify brain areas that are significantly activated during each phase, in remitted depressed volunteers.

One of the brain areas that presented with increased activation during the anticipation phase of the MID task was the SMA (Figure 12). The SMA is involved in cue related processing and is also important for internal generation and preparation of motor activity. In our analysis the SMA presented with increased activation during the anticipation phase of trials that could actually bring monetary rewards, compared to the neutral ones, this would indicate the increased engagement of the participants during reward related trials compared to neutral trials. This finding is in line with previous research for this task, showing that the MID is also a motor task and as such it produces activations in the primary motor cortex, somatosensory areas as well as the SMA (Knutson et al., 2000). That increased engagement to the task could also be interpreted as increased motor preparation for upcoming responses which are controlled by the SMA (Forstmann et al., 2008). The SMA also appeared with increased activations when the anticipation phase of high win trials was compared to low win trial anticipation (Figure 13), this finding further underlies the specific role of that area during the "win" trials of the MID task were a monetary reward could be obtained.

The thalamus is another brain area that presented with increased activation during reward anticipation of the MID trials associated with an actual monetary reward (high win and low win trials) compared to neutral trials (Figure 12). The thalamus along with the NAc and the insula are the three main brain regions that form the reward neurocircuitry (Cho et al 2013). The reward neurocircuitry is responsible for translating motivation into motor responses. Meta-analyses of reward processing in healthy volunteers have revealed that these areas are equally involved during the anticipation of a win or loss (Oldham et al., 2018; Wilson et al.,2018) and also during the anticipation and successful outcome of reward trials (Oldham et al., 2018) and present with increased activations for these contrasts compared to the anticipation and feedback of neutral MID trials.

The significant involvement of those brain areas both during anticipation and feedback could lead to the conclusion that they have a broader role during reward processing in general, and their activation is not dependent on the phase of the task. Another potential role for the thalamus during the MID task is related to the fact that this brain area acts as a hub, processing and transmitting information to and from several neuronal pathways.

The thalamus, with its role as a hub, receives strong input from the visual system and this input plays an important alerting role for salience detection (Usrey and Alitto, 2015). Through the thalamus-striatal pathway, the thalamus could relay the visual information to cholinergic neurons that could create a substrate for the suppression of ongoing motor activity in the face of salient external cues such as the ones that are presented during the MID task (Cho et al., 2013, Haber and Knutson, 2010). As a result, the thalamus has a critical function to adjusting behaviour to incentives. Since the high and low reward MID visual cues have a higher incentive compared to the neutral cues, that area might present with increased activations in order to monitor the participants' behaviours and prevent any premature responses. This role of the thalamus could be considered in conjunction with the increased activation of the SMA which was present during reward anticipation of high and low win trials, compared to neutral trials but also during high reward anticipation compared to low reward anticipation. The increased activation of the thalamus could help control the motor preparation, signalled by the SMA and thus potentially ensure accurate performance of the MID task.

During the feedback phase of unsuccessful trials, irrespective of the magnitude of the lost reward, frontal as well as occipital brain regions along with inferior temporal areas appeared with significantly increased activation (Figure 15). These areas have been linked in the literature with attentional processing (Arrington et al., 2000). Unsuccessful MID trials could be the result of a missed response, when volunteers do not respond at all to the target, or they could be the outcome of a not fast enough response to the target. Missed responses have been excluded for the analysis and as a result the brain activations that are associated with unsuccessful trials arise from the volunteers' slower reaction to the presentation of the target. Slower reaction to the target could be the result of reduced attention to the task.

When feedback is received about trials that were not successful, the increased activation in occipital and temporal areas could reflect the participants increased attention to the task, as a response to the negative feedback that they have received. A similar cluster of areas was activated during the feedback of successful high and low win trials compared to the neutral ones (Figure 16). However, during this contrast these brain areas presented with decreased activations for high and low win feedback compared to the neutral feedback. As mentioned before the activation of these brain areas is associated with attention (Arrington et al., 2000). It is also possible that during feedback for successful trials, despite their reward magnitude the attention that our volunteers pay to the task is decreased as they relax their cognitive efforts in order to perhaps enjoy the obtained reward.

3.1.2 The role of the predefined ROIs to the MID task

Several of the brain areas that were significantly activated during reward anticipation and feedback of the MID task in the whole brain analysis have been previously identified in the literature as important for this task. However, key regions of the reward processing network, do not present with significantly increased activations in our data from the placebo session. These key regions include striatal areas, the amygdala, and the insula which were part of the clusters that presented with increased activations during reward anticipation and magnitude of reward, but their activations do not exceed the significance threshold. The position of these areas in the brain is associated with an increased signal drop out during fMRI which is mainly localized around the striatum which could be contributing to the absence of these areas for our significant results, in the whole brain level. For this reason and because these are areas we know to be involved in this task, we applied an *a priori* defined ROI analysis for these areas allowing us to investigate the changes in activation of these regions during reward processing and anticipation of the MID task.

The *a priori* ROIs that we have chosen to examine as part of our MID analysis are areas that are associated with reward prediction processing and are also part of the salience network. The study of the neuronal mechanisms involved in reward processing, mainly in primates, revealed that reward prediction is mediated by dopaminergic neurons in the striatum (Schultz, 2016b, Schultz, 2016a, Schultz et al., 1993). Moreover, during the different phases of the task, the salience network is activated, which directs the individual's attention towards reward-associated stimuli. The amygdala and the insula are part of that network and along with the VTA and the regions of the ventral and dorsal striatum, comprise the ROIs we chose for this analysis (Izquierdo and Murray, 2007).

Several of our *a priori* defined ROIs presented with significant activations for the different contrasts of the MID task that have been examined in the placebo session (see Table 11). Specifically, during reward anticipation of low and high win trials, compared to neutral trials, the activation of the insula, the putamen and the VS were increased. Moreover, increased involvement of the VTA and VS was also identified when the anticipation phase of high win trials was contrasted to that of low win trials.

Several meta-analyses of fMRI studies that have investigated the neuronal processes that underlie reward anticipation and feedback, for both successful and unsuccessful trials, have concluded that anticipating as well as receiving a reward involves engagement of the VS (for meta-analysis results see (Oldham et al., 2018)). This brain area is involved in reward prediction, during the anticipation of the expected reward but is also implicated in positive or negative prediction error that arises from the feedback phase of successful or unsuccessful trials respectively.

Although the role of the VS in reward processing is very well established, other finding suggest that this brain area could be ascribed a much broader function which also extends to motivational processing. In our data, VS activation appears to be increased during the anticipation of both high as well as lower in value monetary rewards. This finding is in line with animal and human research indicating that the VS, and especially the NAc core, is associated with motivation related functions such as behavioural activation, exertion of effort and sustained task engagement that are independent of the valence of the expected reward (Salamone et al., 2016).

Many of the above described actions, especially sustained attention and engagement to the task are essential during performance of the MID. In our data, whole brain analysis revealed that brain areas associated with attention are activated during the feedback phase of unsuccessful trials (Figure 15). This finding indicates that sustained attention could be essential for successful task performance. Thus, the increased activation of the ventral striatum during anticipation of both high and lower value rewards, could be contributing to successful performance to the MID, as indicated also by the behavioural data of the task (see Figure11) .

In our data VS activity also appeared to be increased during the anticipation phase of high win compared to low win trials in the placebo group (see Table 11). As mentioned before, the VS and especially the NAc core, have a central role in encoding the motivation salience of stimuli (Mannella et al., 2013). MID trials that are associated with higher monetary rewards carry a greater salience value compare to low win trials and thus activation in brain areas that are associated with motivational processing is expected to be increased. The VS and especially the NAc, is also thought to mediate cognitive functions such as cognitive control and effort that are important for the MID (Haber et al., 2010). The fact that the VTA also appeared with increased activation for this contrast is not surprising since the neuronal processes that are important for motivational processing and depend on the VS could be directly modulated by the VTA. This modulation occurs via dopamine producing neurons that initiate from the VTA and project to the VS. These neurons modulate VS activity during the anticipation of both loss as well as gain (Carter et al., 2009).

During the feedback phase of the MID task and when unsuccessful low win trials were compared to feedback from neutral trials, the VTA presented with increased activation for the placebo session (see Table 11). The VTA is a brain area with an important role in the motivational aspects of reward processing and is also functionally and anatomically connected to the VS (Carter et al., 2009). A common finding in studies with depressed individuals who perform anhedonia related tasks, such as the MID is the blunted responses of these individuals to feedback. The blunted response to feedback in depression has been associated with reduced activations in the VS (Pizzagalli et al., 2008, Stoy et al., 2012), an area that is directly modulated by the VTA (Carter et al., 2009).

In our study the MID is performed by remitted depressed volunteers who although recovered from depression might still present, with some of the characteristics of the illness. If a reduced response to feedback which is associated with reduced motivation and effort to obtain a reward still persists during remission, this would be much easier detectable in low reward trials. These trials would have a lower prediction value, compared to the high reward trials, in which the increased value of reward might be sufficient to overcome any deficit. It is thus possible that in our cohort of recovered depressed individuals the increased activation of the VTA would contribute to increased motivational processing that is required to overcome the reduced response to feedback and successfully perform to the task.

3.2 The Effects of ketamine's on the MID task

3.2.1 Whole Brain Analysis

Ketamine's effects on the MID task were first examined on a whole brain level. Reward anticipation, feedback as well as reward magnitude were compared between the ketamine and placebo sessions and it was shown that ketamine did not produce any significant changes in brain activation, 2h after its administration at a whole brain level.

3.2.2 The role of the predefined ROIs to the MID task

When we examined the drug effects on our *a priori* identified ROIs during the different task conditions, ketamine produced significant changes in the activation of those ROIs. Specifically, significant changes were identified, for the ketamine session compared to placebo, both during the reward anticipation as well as the feedback phase of the MID trials. However, ketamine's effects 2h after the administration of the drug seem to be more prominent during the feedback phase of the MID.

3.2.2.1 Ketamine's effects on the task- Reward anticipation

During the anticipation of low win compared to neutral trials, we found that ketamine significantly increased the activation of the caudate, compared to placebo (Figure 17). In the literature the caudate is involved in signalling the reward magnitude of specific cues. Specifically, previous studies (Lutz et al., 2012) which focused on the close inspection of the time-course of activation of this region during the different phases of the MID task, revealed that the caudate is involved in reward processing but representing not only the gain or loss of a reward but also reward magnitude. Moreover, animal as well as human research has shown that the caudate, as part of the dorsal striatum, receives various input associated with reward and feedback from cortical regions (VMPFC, OFC, ACC) as well as limbic areas including the amygdala and the hippocampus. This input is integrated at the caudate and translated into action via signalling to the basal ganglia (Haber and Knutson, 2010) . The increased activation in that striatal region bilaterally, 2h after ketamine, could thus help increase the reward magnitude of cues associated with lower monetary rewards and perhaps facilitate correct responses to these cues

In depression literature, the two meta-analyses that have looked at reward processing in depressed individuals have consistently identified decreased activations in the caudate of those patients relative to healthy individuals. Specifically, when the MID task was examined, the reduced caudate activity was present both during reward anticipation and feedback in patients with MDD (Keren et al., 2018, Zhang et al., 2013). Given the importance of the caudate in reward processing and its altered function in depression which is associated with blunted responses to feedback and anhedonia that area would be a good target for improving anhedonia in MDD. The increases in the activation of the region that we observe, 2h after ketamine, when the antidepressant effects of the drug are detectable, could indicate a possible mechanism for ketamine's positive effects on anhedonia.

3.2.2.2 Ketamine's effects on the task- Feedback

When the feedback phase of successful and unsuccessful trials was examined 2h after ketamine administration we found that during successful trials, ketamine increased the activation in the insula and VS for low win compared to neutral trials (Figure 18). The VS as mentioned before, is an area with very broad function during reward processing as well as motivational processing and presents with decreased activation in depressed as well as remitted depressed volunteers (Mannella et al., 2013, Haber et al., 2010). Specifically, during remission from depression, volunteers presented reduced VS responses to primary rewarding stimuli, compared to healthy controls. These reductions in VS activity have been associated with reduced response to positive feedback in depression (Must et al., 2013) and increased risk for relapse in remission (McCabe et al., 2010). Antidepressant medication has been shown to normalise striatal activations, indicating that reward related deficits could be reversed after treatment (Stoy et al., 2012).

Given the role of the VS in depression and in remission, as well as the fact that this area is a target for antidepressant treatment action, we could hypothesize that the ketamine-induced increases in the activation of the VS would have a positive effect on the feedback processing of successful MID trials. The fact that these increases were observed in our cohort of remitted depressed volunteers, would further underline, the proximity of reward related processes in remission with that observed in depression.

Another ROI that presented with increased activation during the feedback phase of unsuccessful low win trials compared to neutral trials, when the ketamine and placebo sessions were compared, was the VTA (Figure 20). The VTA which also presented with increased activation in the placebo group for the same contrast, is thought to play an important role in signalling prediction errors via dopamine neurotransmission . Moreover, along with the NAc, they have a central role in representing the motivational and affective valence of unanticipated outcomes (Carter et al., 2009). Specifically, a modulatory role of the VTA has been identified in shaping memory, since increased activation of that brain area has been positively correlated with improved recall for stimuli associated with greater potential rewards (Adcock et al., 2006).

The increased activation of the VTA in the placebo group during the feedback phase of unsuccessful low win trials was associated with the increases motivational salience that might be attributed to these trials in order to ensure adequate performance to the task. The ketamine induced increase that is observed in that area 2h after the drug administration and under the same task contrast, could indicate that ketamine would also works towards increasing the salience of those low win unsuccessful trials, making the VTA another possible target for ketamine's delayed effects that could improve anhedonia.

4 Conclusions

Ketamine, 2h after its administration, produces significant changes in the activation of brain areas that are associated with reward processing and the MID task, in remitted depressed volunteers. At this timepoint in the course of our study, when the changes in brain activations are detected, the dissociative and psychotomimetic side-effects that are associated with acute ketamine administration and might be experienced by some participants have completely been resolved. Moreover, while the drug is still present in the blood, ketamine's concentrations are very low any thus any effects on neural processing that ketamine might produce, could be attributed to its active metabolites (Zanos et al., 2018) as well as the remodelling of neuronal architecture induced by the drug (Li et al., 2017) and have been associated with the antidepressant effects of the drug

Despite the neuronal and molecular mechanisms that underlie those effects, ketamine's delayed effects on the MID task, demonstrate as increases in the activity of striatal areas, that are important for reward processing and motivation. Specifically, during reward anticipation ketamine increased the activation of the caudate when low win trials were contrasted to neutral trials and the ketamine session was compared to placebo. For the feedback phase of the MID task, significant increases were identified after ketamine, for the insula and the VS, when the feedback phase of unsuccessful trials was compared to that of successful trials. Finally, the VTA presented with increased activation, when ketamine was compared to placebo, for the feedback phase of unsuccessful low win trials compared to neutral.

In our study, ketamine's effects on the MID task are more prominent during the anticipation and feedback phases of low win trials as well as the feedback phase of unsuccessful trials. Altered reward processing in depression has mainly be associated with decreased motivation as well as blunted responses to feedback (McCabe et al., 2010, Pizzagalli et al., 2008, Treadway and Zald, 2011, Zhang et al., 2013). The remitted depressed volunteers that took part in our study might still present with some of the altered brain activations in reward related areas, that is associated with depression. This could explain why ketamine's effects are more prominent in low win and unsuccessful trials since these trials would be associated with reduced reward magnitude and could require increased effort and motivation and could be associated anhedonia.

Finally, most of the ketamine induced increases in brain activity that we observe in our cohort occur in ROIs of the reward processing network that have presented with decreased activation in depressed patients, compared to healthy controls, when the MID was examined(Keren et al., 2018, Zhang et al., 2013). Some of these areas have also been previously identified as antidepressant medication targets that include the VTA, the VS and the insula (McCabe et al., 2010). These findings further indicate that ketamine's delayed effects in our study could be directly related to the drugs antidepressant mechanisms and also highlight those brain areas as potential targets for ketamine's positive effects on anhedonia.

The VAMP

1 Introduction

Overgeneral AM recall is one of the most common characteristics of depressed individuals. Several brain areas have been identified in the literature as important for AM. However, it remains unknown whether the valence of the recalled memories would influence the brain areas that are activated during AM retrieval. Moreover, very little is known about the early antidepressant effects of ketamine on brain areas such as the amygdala, the ACC and the PCC that are considered key for AM and are also affected in depression.

For the purpose of this study we have developed the VAMP task, a personalised AM paradigm, which allows participants to recall and ruminate about autobiographical positive, negative and neutral life events in the MRI scanner. One component of this task involves rating, using a VAS scale, how positive or negative individuals feel thinking about the events at the time of their participation to the study. This way we can study not only AM recall and rumination but also the effects that the subjective emotional valence of the recalled memories could have on brain areas that are activated during the task. In the chapter that follows we examined the brain activations produced during the VAMP in order to identify the brain areas that are activated during recall of positive, negative and neutral AMs, in the placebo group.

This analysis will help us determine which brain regions are significantly activated during general AM recall, in remitted depressed volunteers and examine whether the valence of the AMs alters the brain areas that are activated during their recall. Moreover, we used a PPI analysis to explore the connectivity, during the different task contrasts. Using *a priori* identified ROIs which include the bilateral amygdala, ACC and PCC we compared the brain connectivity during positive, negative and neutral AM recall in the placebo group.

Ketamine's effects on the VAMP, 2h after the drug administration, were first examined by looking at the VAS ratings for each event. These rating were compared to those obtained during the placebo session in order to examine whether ketamine would significantly alter the subjective emotional valence of the AM events that are recalled during the task. Furthermore, by examining the different task contrasts between the ketamine and placebo sessions, we investigated whether ketamine could significantly change brain activity during AM recall of positive, negative and neutral events.

Using the PPI approach, we examined the effects of the drug on task connectivity. Specifically, we investigated whether ketamine, 2h after its administration, would significantly alter the connectivity between our pre-selected ROIs and the rest of the brain, compared to placebo. Finally, we used the VAS ratings from the two study sessions to examine whether brain activity during positive, negative and neutral recall would correlate with those ratings.

2 Results

2.1 Behavioural Analysis

Analysis of the VAMP task started by looking at how many statements were recognized as "TRUE" by our participants. During the decision phase of the VAMP task as described in our methods (see page 76), participants have 6 secs to decide whether a statement referring to a particular event is "TRUE" or "FALSE .Those participants who failed to respond within that 6 sec window and/or falsely identified more than 20% of the statements from each study session (6 statements out of a total of 30 statements) were excluded from the analysis as it was deemed that they were not paying sufficient attention to the task. Based on that cut off all participants were included in the analysis.

In order to get an understanding of the subjective emotional valence of the three types of events, positive negative and neutral, that were examined with the VAMP task, we averaged separately, the VAS ratings that were obtained for each statement belonging to each event type. In our average we included only ratings from statements that were recognised as "TRUE" from the participants and were actually true, according to the individuals' original description of the event on the interview day. Moreover, any ratings of positive statements that were below 60, any rating of negative statements that were above 40 and any ratings of neutral statements that were below 40 and above 60 were excluded from the analysis. Although these cut-offs are arbitrary, we believe that these statements would not be representative of the valence of the event types that they belong to. These statements were also excluded from the fMRI analysis.

Moreover, the individual ratings from the positive and negative event form each participant, were compared to the neutral average and any statements from those events (positive and negative) that were rated within 2SDs from the neutral average were excluded. The reason for that is that we believe that participants would consider them neutral at the time of the scan. Only three statements from each different volunteer were excluded based on that.

2.2 Neuroimaging results

2.2.1 Whole brain analysis of AM recall and rumination in the placebo session

The fMRI data analysis included only statements that were identified as "TRUE" by our participants and were actually true, according to the volunteers' original narrative of the events during the interview day. The retrieval phase from these events was then used to examine brain activity under different contrasts and compare it between the ketamine and placebo sessions.

2.2.1.1 Positive, negative and neutral events compared to the control condition

In order to identify the brain areas that are important during active recall of the VAMP events, compared to the task's control condition, we examined the retrieval phase of true statements of all events (positive, negative and neutral) and contrasted them with the control condition. During active recall, several brain areas that have previously been identified as important for AM retrieval in the literature present with significantly increased activations compared to the control condition.

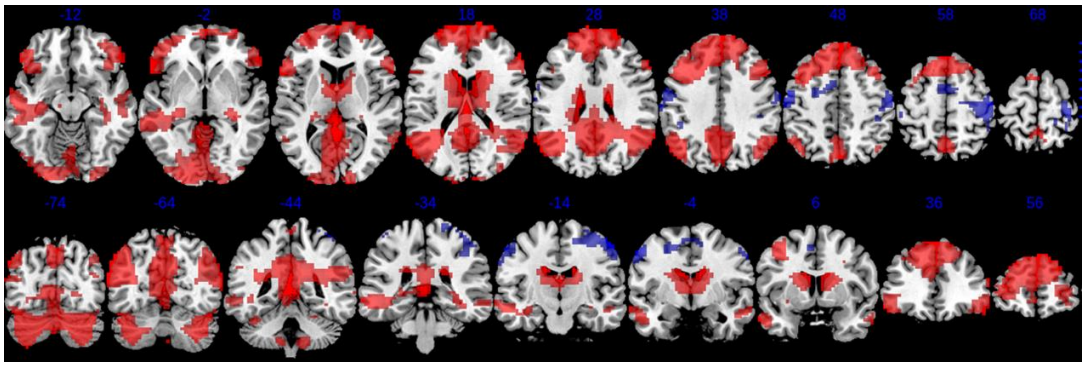

Figure 22. *Whole brain analysis of the VAMP in the placebo group revealed significant increases (red) and decreases (blue) in the activation of several brain areas, during recall of positive, negative and neutral memories compared to the control condition.*

Cluster level pFWE_corr	Peak level pFWE_corr	Brain Region
0.000	0.000	Front_Mid (-45 19 46)
0.000	0.000	Precuneus (-8 -60 33)
0.032	0.001	Cerebellum (19 -75 -33)
0.063	0.010	Postcentral (-52 -8 49)
0.000	0.014	Thalamus (11 -15 10)

Table 13. *Active retrieval during the VAMP task, compared to the control condition revealed increased activations in three clusters and siginificant decreases in two. The MNI coordinated for the peak of each cluster are also presented. The valence of the recalled memory in the Placebo group*

When we compared the retrieval phase of positive and negative memories to that of neutral memories and we did not identify any significant changes in brain activity in the placebo group. No significant changes were also identified when we compared the retrieval phases of the positive to the negative memory trials. These findings could indicate that the brain areas that are important for AM recall during the VAMP, do not change when the emotional component of the recalled memory changes.

2.2.2 PPI analysis of the amygdala connectivity during the placebo session

PPI analysis allowed us to investigate the connectivity between our seed regions and the rest of the brain, under the different task conditions. For the purpose of this analysis we looked at the retrieval phase of the positive and negative AMs and compared it to the retrieval phase of the neutral memories. From the three seeds that were *a priori* defined for this analysis and included the bilateral amygdala, the PCC and sgACC only the bilateral amygdala showed significant connectivity with other brain regions during the task.

2.2.2.1 Retrieval phase of positive AM recall compared to neutral AM recall

Specifically, in the placebo group and during recall of positive AMs compared to neutral, the amygdala showed increased connectivity, bilaterally, mainly with the lateral occipital cortex, the precentral gyrus and the occipital/fusiform gyrus. Decreased connectivity was observed, during positive AM recall compared to neutral, between the bilateral amygdala and the bilateral superior and right middle temporal gyrus, the right hippocampus, the putamen and the insula, bilaterally. The results from this analysis are show in Figure 23.

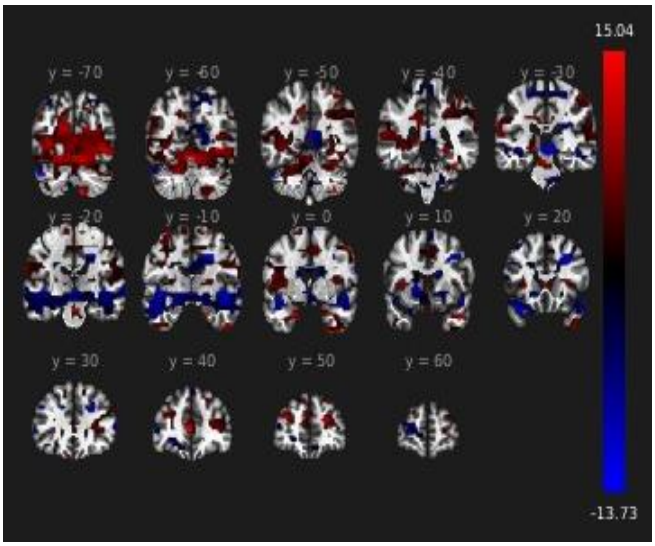

Figure 23. *Significant changes (p<.05, FDR corrected) in the connectivity betweem the bilateral amygdala and several brain areas were identified when the retrieval phase of the positive and neutral events was examined in the placebo group. The brain areas where connectivity with the amygdala increased (red) included a. the precuneus b. the precentral gyrus(bilateral) c. the lateral occipital cortex (bilateral) d. the post central gyrus (bilateral) e. the occipital pole (bilateral) f. the temporal pole (bilateral) g. the cerebellum-Crus1(left) h. the insular cortex (bilateral) i. the thalamus (right) j. the occipital fusifrom gyrus (bilateral) k.the putanen l. the hippocampus (right). Brain areas where connectivity with the amygdala decreased (blue) included: a. the parahippocampal gyrus (bilateral) b. the paracingulate gyrus (bilateral) c. the intracalcarine cortex (bilateral) and d. the occipital pole (left) d. the middle temporal gyrus (right) e. the superior temporal gyrus (bilateral) f. the hippocampus (left) g. the caudate (bilateral) h. insula (bilateral) and i. the puatmnen*

2.2.2.2 Retrieval phase of negative AM recall compared to neutral AM recall

When the retrieval phase of negative AMs was compared to that of neutral AMs, increased connectivity was identified between the bilateral amygdala and middle temporal gyrus, the orbital frontal cortex, the lateral occipital cortex, the fusiform gyrus, the thalamus and posterior and anterior cingulate cortex. Decreased connectivity was identified between bilateral amygdala and the hippocampus, the thalamus, the middle and superior temporal gyrus, the angular gyrus and the caudate. The results of this analysis can be seen in more detail in Figure 24. All these significant increases and decreases in the connectivity between the amygdala and the rest of the brain, during negative AM recall compared to neutral, occurred bilaterally.

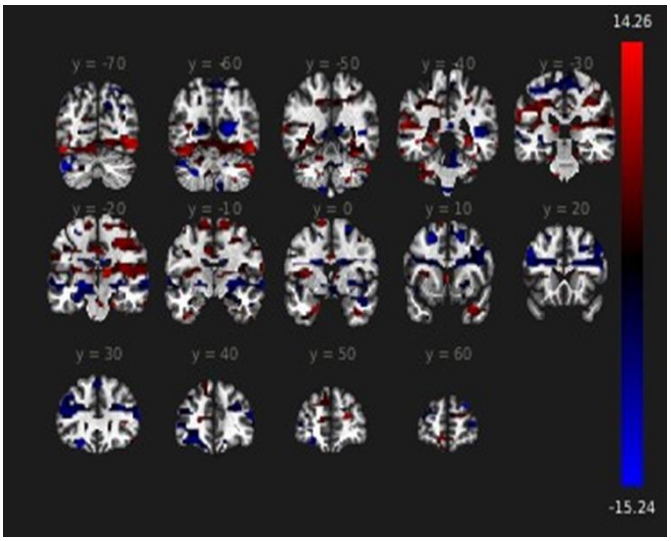

Figure 24. *Brain areas that present with increased (red) and decreased (blue) connectivity with the bilateral amygdala during recall of negtive compared to neutral events are presented (p<.05 FDR corrected) in this figure. Significant increases in connectivity were identified in the a. orbital frontal cortex (bilateral) b. middle temporal gyrus (bilateral) c. precuneus (bilateral) d. middle frontal gyrus (bilateral) e. inferior frontal gyrus (bilateral) f. the cerebellum-Crus2 (left) g. the superior temporal gyrus (bilateral) h. the lateral occipital cortex (bilateral) i.the occipital fusifrom gyrus (bilateral). Significantly decreased connectivity were identified between the bilateral amygdala and a. the thalamus (bilatral) b. the hippocampus (bilateral) c. the parahippocampal gyrus (bilateral) d. temporal pole (bilateral and e. the parahippocampal gyrus (bilateral).*

2.2.2.3 *The effect of emotional valence on the VAMP*

In order to investigate the effect of emotional valence, connectivity of the bilateral amygdala with the rest of the brain was examined in the placebo group. For this analysis the retrieval phase of the positive AM trials was compared to the retrieval phase of negative AM trials. Significantly increased connectivity was observed between the bilateral amygdala and the temporal/occipital fusiform gyrus, the lateral occipital and middle temporal cortex as well as the SMA for recall of positive memories compared to negative ones. The inferior temporal gyrus, the thalamus, the hippocampus, and the left inferior frontal gyrus presented with decreased connectivity with the bilateral amygdala for the aforementioned contrast. Figure 25 shows the results from this analysis. Unless specified, all these significant increases and decreases in the connectivity occurred bilaterally.

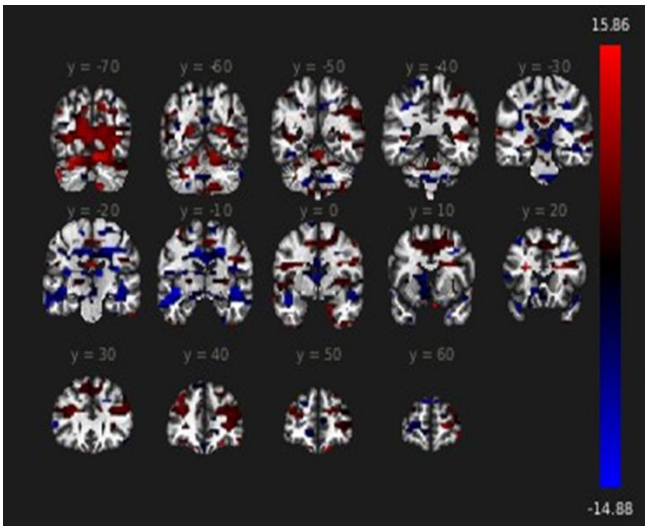

Figure 25. *When the effect of the valence of the recalled memory was examined, significant (p<0.05, FDR corrected) increases and decreases inconnectivity of the bilateral amygdala with the rest of the brain were identified. Increased connectivity was identified between our seed region and a. the lateral occipital cortex (bilateral), b. the precuneus (bilateral), c. superior frontal gyrus (bilateral), d. temporal and occipital fusiform gyrus (bilateral), e. the SMA (bilateral) f. middle temporal gyrus. Significant decreases were identified for a. the thalamus (bilateral), b. the hippocapus (bilateral), c. the inferior frontal gyrus (left) and d. the inferior temporal gyrus (bilateral).*

2.2.3 The effects of ketamine on task performance

The VAS ratings of the positive, negative and neutral statements were averaged and compared between the ketamine and placebo sessions. The purpose of this analysis was to examine whether ketamine, compared to placebo, would produce any significant changes in the emotional valence of the events, as it is perceived by our participants at the time of the scan. No significant changes were identified between the VAS ratings of the ketamine and placebo sessions. The mean ratings for each event and each session are presented in Figure 26 and the VAS ratings for each event type are includes in Table 13.

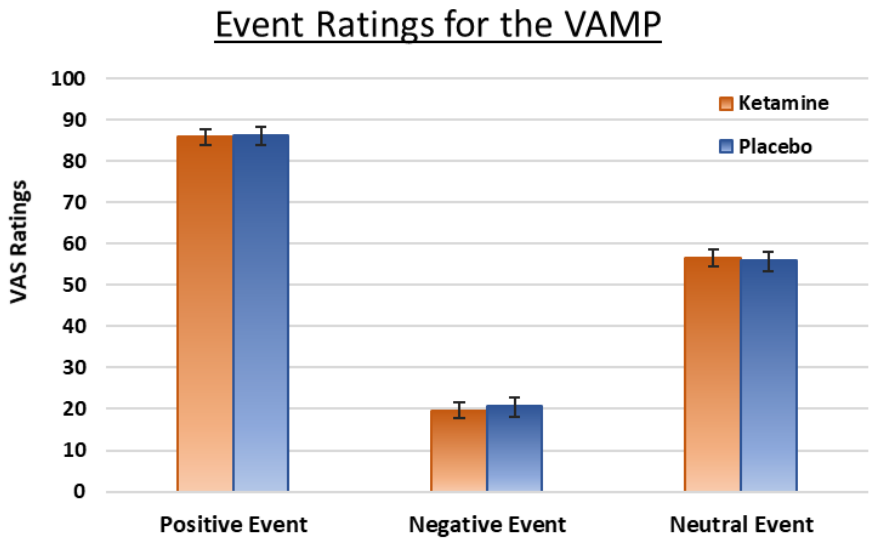

Figure 26 *The average VAS ratings of the positive, negative and neutral events. A paired t-test revealed that the ratings did not change between the ketamine and placebo sessions (p>0.05)*

	Mean	N	Std. Deviation	Std. Error Mean
Pos_ket	85.80	36	9.85	1.93
Pos_plcb	86.26	36	10.85	2.13
Neg_ket	19.70	36	12.55	2.46
Neg_plcb	20.65	36	13.78	2.70
Ntr_ket	56.58	36	7.02	1.38
Ntr_plcb	55.91	36	7.81	1.53

Table 14. *The mean VAS score for the positive, negative and neutral event did not differ (Paired t-test, p<.05) between ketamine and placebo, 2h after the drug administration.*

2.2.4 PPI analysis of the amygdala connectivity between the ketamine and placebo sessions

2.2.4.1 Retrieval phase of positive and negative recall compared to neutral

Ketamine, 2h after its administration, disrupted the connectivity patterns that were identified in the placebo group, between the amygdala and several brain regions. Specifically, during recall of positive memories compared to neutral (Figure 27A), increased connectivity was observed between the bilateral amygdala and temporal regions including the middle and inferior temporal gyri and the temporal pole but also the precuneus and the precentral gyrus. Decreased connectivity was identified between the seed region and the lateral occipital cortex as well as the occipital fusiform gyrus and the parahippocampal gyrus. Some of these regions, mainly the lateral occipital areas, presented with increased connectivity with the amygdala when the same contrast was examined in the placebo group.

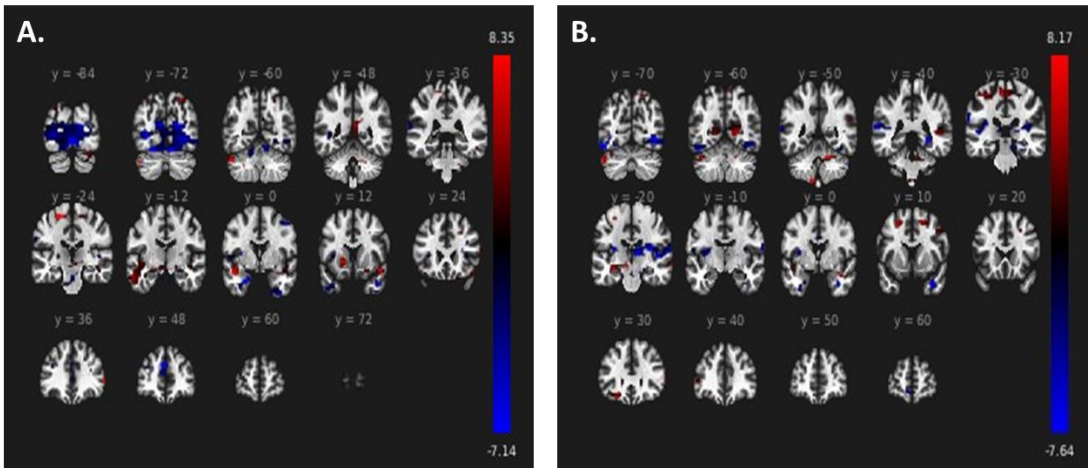

***Figure 27**. A. Ketamine, 2h after its administration significantly increased the connectivity between the bilateral amygdala and a. the temporal pole (bilateral), b. middle temporal gyrus (bilateral),and c. the inferor temporal gyrus (bilateral)d. the precuneus (bilateral) f. precentra gyrus (bilateral) during the retrieval phase of **positive memories** compared to neutral. Significantly decreased connnectivity was identifies between our seed region and a. the occipital fusiform gyrus (bilateral), b. the parahippocampal gyrus (bilateral) c. the occipital pole (bilateral) d. the insular cortex (bilateral). B. When the connectivity of the amygdala was compared between the ketamine and placebo sessions for the retrieval phase of **negative memories** compared to neutral, significant increases were identifies for our seed region and a. precuneus (bilateral), b. the frontal occipital cortex (bilateral), c. middle and inferior frontal areas (bilateral) d. the middle and superior temporal gyrus (bilateral). Decreased connectivity during negative memory retrieval was identifies between the amygdala and a. the insular cortex (bilateral), b. the parahippocampal gyrus (bilateral), c.the hippocampus(left), d.the thalamus (bilateral), e.the fusiform gyrus (bialteral), f. the lateral occipital cortex (bilateral).*

The same pattern of disrupted connectivity between the amygdala and occipital and temporal areas was also observed when the retrieval phase of negative memories was compared to neutral recall between the ketamine and placebo sessions (Figure 27B). Ketamine, 2h post the drug administration, increased connectivity between the bilateral amygdala and the superior temporal gyrus, the middle temporal gyrus and the frontal occipital cortex. Decreased connectivity, however, was observed for the same contrast, between the amygdala and lateral occipital cortex, the fusiform gyrus, the thalamus and the left hippocampus. Unless specified, those significant changes in the connectivity of positive and negative AMs, between the ketamine and placebo sessions, occurred bilaterally.

2.2.4.2 The effect of emotional valence of recalled memories on task connectivity

When the effect of valence was examined, and positive AM retrieval was compared to negative AM retrieval, ketamine did not produce any significant changes in the connectivity of the amygdala with the rest of brain.

2.2.4.3 The effect of session

Our relatively large sample size (N=36) allowed us to examine whether the order of drug administration (placebo on the 1st session or ketamine on the 1st session) would affect the performance as well as the brain activations observed during the VAMP. For that purpose, the retrieval phase of positive, negative and neutral AM trials was examined in an analysis that included only participants who were administered ketamine on the 1st session. These connectivity results were compared with those obtained for those participants who received placebo on the 1st session. A total of 18 participants were included in the two groups that were created for this between-subjects analysis and all the contrasts of interest that were examined for the VAMP task as well as the PPI analysis were repeated.

Ketamine did not produce any significant effect of session on the VAMP compared to placebo.

3 Discussion

Summary of Findings

The VAMP task has been specifically developed in order to examine ketamine's delayed effects on brain areas that are activated during AM recall and rumination. Since the VAMP is a novel variant of autobiographical memory tasks that were previously used in research, we first examined the data from the placebo session in order to identify the brain areas that are engaged by the task. Using a whole brain approach, the positive, negative and neutral memories that are recalled during the task, were compared to the control condition and also contrasted to each other. This analysis lead to the identification of those brain regions that are important for active recall and rumination (see Figure 22). Several of the brains regions that presented with significant activations during the positive, negative and neutral AM trials, compared to the control condition, have been previously discussed in the literature for their important role during AM recall.

The examination of brain areas, at a whole brain level, that are activated during the retrieval phase of AM trials with different emotional valence (positive vs negative, positive vs neutral and negative vs neutral) lead to the conclusion that during the VAMP, the same brain areas are activated for positive and negative memory recall. A task-based connectivity analysis revealed that the bilateral amygdala presented with significantly increased and decreased connectivity with several brain areas when positive and negative AM recall was compared to neutral (see Figure 23 and Figure 24). Moreover, this finding was sensitive to the emotional valence of the retrieved memories since the connectivity of the bilateral amygdala with the rest of the brain was significantly different for positive compared to negative AM recall (see Figure 26).

Ketamine's effects on the task, were initially examined, at a whole brain level, and it was shown that ketamine, 2h after its administration, did not produce any significant changes to the task activations, compared to placebo. Moreover, the emotional valence of the recalled memories did not significantly change between the ketamine and placebo sessions when the VAS ratings were compared under the different drug conditions (see Figure25 and Table 13).

Finally, in order to investigate whether ketamine would alter the task-based connectivity between our seed region and the rest of the brain we repeated the PPI analysis and compared the ketamine with the placebo sessions for all the contrasts of interest. We found that ketamine produced significant disruptions in the connectivity between the amygdala and the occipital cortex both during positive as well as negative AM recall (see Figure 27).

3.1 The VAMP task- placebo group

AM memory retrieval involves the accurate reconstruction of mental representations of past events by associating together the details that are related to the retrieved event (Sheldon et al., 2019). Specifically, during AM recall, mnemonic processes are activated to help recreate a detailed memory representation by associating different types of information, processed by disperse neuronal systems such the auditory and visual networks (Greenberg and Rubin, 2003). Previous neuroimaging studies of AM recall have shown that during this complex and cognitively demanding process, an extensive network of cortical and subcortical brain areas are activated in order to contribute to an accurate reconstruction of the retrieved AM (Dolcos et al., 2012, Wheeler et al., 2006). Moreover, there is evidence supporting an extensive overlap between the encoding and retrieval AM networks, since key regions that are implicated in AM recall, such as the amygdala, the hippocampus and parahippocampal areas along with the visual cortex are also activated during AM encoding (Kim, 2019, Kim et al., 2010).

In our fMRI analysis of the VAMP task several of the brain areas that form part of the AM retrieval network presented with increased activations during the recall of positive, negative and neutral memories, compared to the control condition (Figure 22). Among the areas that presented with significantly increased activation during AM recall, the precuneus has been extensively discussed in AM literature. The precuneus is located in the posterior-medial portion of the parietal lobe and is considered important for the accurate reconstruction of the visual-spatial imagery associated with memory recall (Fletcher et al., 1995, Cavanna and Trimble, 2006).

As part of the DMN, the precuneus is also activated during self-referential processing, and thus is important not only during active memory retrieval but also ruminative thinking (Utevsky et al., 2014). The increased activations observed in that region during the VAMP, could indicate that during active recall of positive, negative and neutral AMs, visual reconstruction of the retrieved memories takes place. However, there is evidence supporting that the activation of the precuneus is necessary for successful memory retrieval, regardless of the imageable characteristics of the retrieved memories (Cavanna and Trimble, 2006). As a result, the increased activation of the precuneus could indicate the successful retrieval of AMs during the task.

Another possible function for the precuneus during AM recall, could be associated with its role within the DMN. The precuneus as part of the DMN, is important for maintaining self-consciousness as well as regulating goal-directed actions (Utevsky et al., 2014, Cunningham et al., 2017, Vatansever et al., 2015). Although traditionally thought to be activated during rest, the DMN presents with increased activations during several AM tasks and is implicated in reward and outcome monitoring paradigms as well as during emotional stimulus processing (Vatansever et al., 2015). Specifically, studies have shown that within the DMN, the precuneus exhibits connectivity patterns that differ from the larger network. It has thus been suggested that this brain area could act as a hub region which links the DMN with other, mainly fronto-parietal brain areas (Utevsky et al., 2014). As a hub region the precuneus could be activated during the VAMP in order to integrate and process information from several networks whose activation is necessary for successful AM retrieval.

Neuroimaging studies that have examined AM retrieval, have consistently shown that temporal areas and more specifically the hippocampus play an important role during memory recall (Buchanan et al., 2005, Greenberg et al., 2005). However, examination of the VAMP task did not reveal significant hippocampal activations during active recall, compared to the control condition. One possible explanation for the lack of significant hippocampal activity during the task could be the relatively long recall window (12seconds) that was given to volunteers in order to retrieve and ruminate about their AMs.

According to a study by Daselaar et al., which aimed to investigate the temporal dynamics of the brain areas engaged in AM retrieval, it was shown that the hippocampus might be essential for the early stages of memory reconstruction but could be less involved once retrieval progresses have progressed further. In the aforementioned study, when participants were given a 10s time-window to think about specific AMs, temporal analysis of the data suggested a shift of brain activity, from an initial activation of the hippocampus, retrosplenial and right PFC to a later recruitment of the visual cortex, the ACC and the PCC (Daselaar et al., 2008). These areas presented with significantly increased activation for active retrieval in our task

During the VAMP, the 12sec retrieval phase is precipitated by a 6 sec decision phase during which the statements from each event are initially presented to participants. The decision phase of the task would require active retrieval of the specific AM in order the volunteers to be able to decide whether the statement is “TRUE” or “FALSE”. Although short in duration, the decision phase of the task might be long enough for an initial reconstruction of the specific AM which would be further elaborated during the retrieval phase of the task. It is thus possible that the hippocampus could be involved during that initial stage of AM recall while areas such as the visual cortex and the precuneus, which are important for the later stages of retrieval, would present with significantly increased activations during the retrieval phase (Daselaar et al., 2008). The very short length of the decision window, which is also accompanied by button presses that vary in time, makes it difficult to examine the brain areas that are important during that phase of the VAMP task and thus validate this hypothesis in our data set.

Another interesting explanation for the absence of hippocampal involvement during AM retrieval in our task, has recently been proposed by Sheldon et al who suggest that the brain areas which are activated during AM recall could vary based on the purpose that drives the memory retrieval. Specifically, there is evidence indicating that autobiographical event information is stored in a hierarchy, at different levels of abstraction and that during recall, information from different levels is assembled to construct the memory (Sheldon et al., 2019). This constructive feature of AMs means that the same AM could be formed in multiple ways, engaging different levels of detail from each hierarchy level which could also be reflected upon the differential contribution of brain areas during recall of the same memory.

When AMs are remembered as conceptual experiences, for example, which could contribute to future planning and decision-making, especially during novel or ambiguous situations (Pillemer, 2003), focus has been given on the emotional experiences that are associated with a specific AM. In that scenario, brain areas that are associated with self-referential processing such as the dorsal medial PFC and the ventral medial PFC, but also the amygdala, that are also involved in emotional evaluation of the memory are activated (Binder et al., 2009, Lin et al., 2016). Conceptual recall also involves the activation of temporal areas and the hippocampus that are implicated in knowledge-based processing (Preston and Eichenbaum, 2013).

During perceptual retrieval, when AMs are retrieved as formed images of experienced events, areas that are important for sensory and perceptual processing are activated including visual processing areas (ex. occipital cortex) as well as somatosensory brain regions (eg somatosensory cortex, precuneus) that would recreate the specific situational elements of an encountered event (Reagh and Ranganath, 2018, Ritchey et al., 2015).

We believe that during the retrieval phase of the VAMP task, when memory recall occurs in a well-structured scenario where there is little novelty or ambiguity as for the purpose of the recall, a perceptual retrieval is more likely to occur. Consequently, there would be greater engagement of brain areas that would contribute to an accurate and detailed representation of the memory such as the precuneus and absence of activation in brain regions that are more involved in conceptual retrieval. Of course, a clear distinction between the brain areas that are engaged in these two types of AM reconstruction is difficult (Addis et al., 2007, Sheldon et al., 2019), especially during our task which was not designed to specifically target perceptual retrieval of past AMs.

3.2 The effects of emotional valence in AM retrieval

During the analysis of the VAMP data, at a whole brain level, and in order to examine whether the emotional valence of the retrieved memory would alter brain activations, we compared the retrieval phase of positive, negative and neutral trials. We found that regardless of the emotional valence of the recalled memory, the same brain areas are activated in the retrieval phase of the VAMP.

The role of emotion in AM encoding and retrieval has been the subject of several fMRI studies. Most of these studies indicate that emotional arousal during the encoding of AMs could activate brain areas such as the amygdala and the OFC which are important for affective processing(Conway, 2005, Kim et al., 2018). The activation of these brain regions could, in turn, boost the strength of the memory by enhancing the activation of regions that facilitate the encoding of sensory detail (fusiform gyrus) and consolidation of memory (hippocampus) (Kensinger, 2009). As a result, emotional memories are more strongly encoded and consequently more easily retrieved. The neuroimaging evidence, however, highlights that emotion specific processes do not replace standard memory networks that are essential for the successful encoding and retrieval of AMs but can only modulate them (Dolcos et al., 2017). These modulations could be rather subtle and thus not easy to capture at a whole brain level.

This finding, however, does not necessarily indicate that the emotional valence of the recalled memory does not influence the connectivity between different brain areas, which could change based on the emotional component of the retrieved AM. For that purpose, we conducted a PPI analysis which would enable us to examine with more sensitivity the connectivity between different brain areas and how this could change based on different task conditions.

Task-connectivity analysis in the placebo group

The role of the amygdala

When the task connectivity was explored during the retrieval phase of positive and negative memories compared to neutral, it was shown that in the placebo group, the bilateral amygdala presents with significantly altered connectivity with several cortical and subcortical regions (Figure 23 and Figure 24). The connectivity analysis using the PCC and the sg ACC did not produce any significant findings. The significant involvement of the amygdala during the recall as induced by the VAMP is not surprising given the central role that the emotional valence of the recalled memories played in the design and implementation of this task.

Autobiographical memories, as mentioned before, are contextually rich memories, and their retrieval would activate brain areas that are important for sensory and emotional processing (Kensinger, 2009, Kim, 2019). The amygdala, a brain area with a central role in emotional processing, is also a pivotal component of the affective network which is important for emotional regulation. The role of the amygdala activation however, during AM encoding and retrieval is rather unclear. In the literature there is some evidence indicating that increased amygdala activity could either enhance (Kensinger and Schacter, 2005) , have no effect (Dougal and Rotello, 2007, Onoda et al., 2009) or even impair (Strange et al., 2003) the formation of certain memories.

Despite the unclear role of the amygdala during AM processing, this brain area has been consistently identified as one that presents with altered connectivity with several other brain regions, in depressed compared to healthy individuals (Young et al., 2018) . Moreover, altered amygdala activation has been shown to persist in remission (Young et al., 2016). As a result, the study of the amygdala connectivity during AM retrieval of emotionally valenced memories, is not surprising and would help us better understand AM recall in our cohort of remitted depressed individuals but also identify any effects related to ketamine's antidepressant action on that brain area.

The amygdalae are functionally and anatomically connected with several brain areas, including the hippocampus and parahippocampal regions, the insulae, the caudate nuclei and regions in the orbitofrontal cortex (Bickart et al., 2014). During the VAMP task, the connectivity between the bilateral amygdala and the rest of the brain was examined for positive and negative memory recall, compared to neutral. This analysis revealed significant increases and decreases in the connectivity between the amygdala and several cortical and subcortical brain areas. The increased connectivity identified between the amygdala and lateral occipital regions involved in visual processing as well as the decreased connectivity between the amygdala and the left hippocampus of particular interest to our study. These findings were present during positive recall compared to neutral and negative recall compared to neutral. The amygdala also presented with decreased connectivity with the insula, bilaterally, during positive AM recall compared to neutral and that is another interesting finding (see Figure 23).

Retrieval of AMs, as already mentioned, involves the emotional re-experience of an event, and this aspect of memory recall is the foundation of episodic memory (Kensinger, 2009, Kim and Moore, 2019). Consequently, it is not surprising that during recall of emotionally valanced AMs brain areas that are important for sensory and emotional processing would be connected to each other. One such area, that presents with increased activation during AM recall (whole brain level analysis) as well as increased connectivity with the amygdala during the VAMP task (PPI analysis) is the visual cortex. The visual cortex has been associated with elaboration during memory retrieval and its activation could be influenced by emotional modulation (Hofstetter et al., 2012). The fact that it appears to be significantly more connected to the amygdala during the retrieval of positive and negative memories compared to neutral, could indicate the stronger interplay between emotional and sensory related brain areas for the recall of memories that are emotionally valenced. It could also point to a more elaborate and accurate recall of those memories.

When the connectivity between the amygdala and the rest of the brain was examined for the retrieval phase of positive as well as negative memories and compared to neutral, decreased connectivity was identified between the seed region and the left hippocampus (see Figure 23 and Figure 24). The amygdala and the hippocampus are adjacent brain areas within the medial temporal lobe and are richly connected to each other. The hippocampus is thought to support the retrieval of AMs with rich contextual input (Maguire, 2001, Svoboda et al., 2006) whereas amygdala activity seems to be mostly related to processing of affectively rich, internal information (Bickart et al., 2014). The interplay between the two brain areas is thought to be important for memory reconsolidation (McGaugh, 2002) and the connectivity between those two brain areas appears to be elevated during negative memory recall in depression. Dore and colleagues using a very similar AM task and this increased connectivity was associated with altered emotional regulation (Dore et al., 2018). The decreased connectivity between the amygdala and the hippocampus that we observe during emotional memory recall, compared to neutral in our task could indicate the emotional regulation that occurs during recall of positive and negative AMs compared to neutral.

The effect of valence

When the effect of the emotional valence of the retrieved memories was examined using PPI, we observed that during the retrieval phase of positive AMs compared to negative AMs, there was increased connectivity between the amygdala and the visual occipital areas (see Figure 26). Since we propose that the functional connection between the amygdala and the visual processing areas reflects the emotional reconstruction of the recalled memory, this increased connectivity could be an indication of the increased cognitive effort required for the successful recall of positive memories in remitted depressed volunteers. We know from the existing literature that the cognitive bias towards negative memory recall along with increased amygdala activations could persist during remission (Young et al., 2018). Thus, it is possible that more interplay between the amygdala and the visual cortex is required for a successful retrieval of positive memories compared to negative ones in our cohort.

Retrieval of positive AMs, in the placebo group, significantly decreased the connectivity between the amygdala and the left hippocampus (see Figure 26). As we mentioned before, connectivity between the amygdala and hippocampus, is important for emotional regulation (Dore et al., 2018). The reduced connectivity between these two areas during recall of positive AMs compared to neutral, could be associated with the reduced emotional burden that positive AM would have on our volunteers. As a result, less emotional regulation would be required during recall of these memories compared to negative AMs.

Ketamine's effects on task connectivity

When the effects of ketamine on the VAMP task were examined, 2h after the drug administration, ketamine did not produce any significant changes in the VAS ratings of the positive, negative and neutral events (see Figure 25). Moreover, when whole brain analysis was used to examine the effects of ketamine on the VAMP task no significant changes were identified in the activation of brain areas that are important for active recall, compared to the control condition. No significant changes in brain activations were identified when ketamine's effects on the valence of the recalled memories was examined.

When VAMP connectivity was examined using PPI, and compared between the ketamine and placebo sessions, significant changes were identified only between the connectivity of the amygdala with the rest of the brain. The most important of these changes included the disruption in the connectivity pattern between the amygdala and the visual cortex, produced by ketamine 2h after its administration. Specifically, the visual cortex presented with decreased connectivity with the amygdala when positive and negative AM recall were respectively contrasted to neutral AM recall and compared between the ketamine and placebo sessions (see Figure 27A and 27B).

The amygdala and the visual cortex appeared with increased connectivity for positive AM recall, compared to neutral in the placebo group and this finding was interpreted as an indication of the increased effort that is required for an accurate and detailed recollection of positive memories compared to neutral in our cohort of remitted depressed volunteers. Ketamine, 2h after its administration, decreased the connectivity between the amygdala and the visual cortex, for negative as well as positive AM recall when compared to neutral. This decrease which appears to be independent of the emotional valence of the recalled memory, could be indicative of the reduced cognitive effort that is required for the accurate recollection of both positive as well as negative memories after ketamine administration.

Another possible explanation for the decreased connectivity between the amygdala and the visual cortex, observed 2h after the drug administration and for the emotionally valenced memories compared to neutral, could be associated with less imagery related to the recall of those memories after ketamine. Recent theories about human memory propose that AM s when recalled, enter a labile state and thus they need to be reconsolidated (Agren, 2014, Nadel et al., 2000). In a study by Schwabe and Wolf (2009), recall of AMs under different contexts, lead to altered details during their subsequent recollection. Although only neutral memories were disrupted in that experiment, that was associated with the increased emotional valence of positive and negative AMs which leads to their stronger encoding, making it more difficult to temper with those memories during reconsolidation (Kensinger, 2009).

If the positive and negative AMs become labile during their recall for the VAMP task, it is possible that ketamine, by altering the imagery associated with those memories could affect the re-encoding of those memories and subsequently their future retrieval. This would be in line with research showing that the visual vividness of memories during encoding, could influence their subsequent retrieval (D'Angiulli et al., 2013). The design of our task, which did not involve any assessment of the quality of memory recall after ketamine and placebo does not allow us to directly validate this hypothesis. If, however, ketamine 2h after its administration, interferes with the reconsolidation processes of emotional memories, this could be provide a neuronal mechanism for its antidepressant action and make ketamine a good candidate for combination treatments that would include cognitive therapies.

Ketamine, 2h after its administration, also decreased the connectivity between the bilateral amygdala and the hippocampus, and this finding was present during negative AM recall compared to neutral. As we mentioned before the interplay between the amygdala and the hippocampus is particularly important for emotional regulation. Depressed individuals present with an increased connectivity between those brain regions during recall of negative memories and distancing strategies could lower the hypeconnectivity between those two areas and help regulate their emotional responses to negative events (Dore et al., 2018). The decrease in the connectivity between the amygdala and the hippocampus, under ketamine, compared to placebo might reflect the reduced cognitive effort required for the emotional regulation of negative memories in that cohort.

4 Conclusions

The VAMP task, that was for the first time tried in this study produced, at the whole brain level and in the placebo group, significant activations in brains areas that are important for autobiographical memory recall. When the connectivity of the amygdala and the rest of the brain was examined it was shown that during recall of positive and negative memories the amygdala presented with increased connectivity with the visual cortex and decreased connectivity with the hippocampus. The interplay between the amygdala and those brain areas could be linked to the visual imagery associated with emotional memory recall as well as emotional regulation processes. These findings indicate these cognitive processes are engaged during recall of emotionally valenced memories when remitted depressed volunteers perform the task.

Ketamine, 2h after its administration, when the drug levels in the blood are low and participants have recovered completely from any psychotomimetic/dissociative effects that the drug might produce, disrupted the connectivity between the amygdala and the visual cortex as well as the amygdala and the hippocampus. This disruption was significant when the retrieval phase of positive and negative memories was separately compared to retrieval of neutral AMs. These findings indicate that the delayed effects of ketamine might be linked to brain areas that are important for emotional memory recall. This could indicate a possible antidepressant mechanism for the ketamine's antidepressant action which be associated with reconsolidation processes that could improve rumination and negative affect.

General Discussion

Introduction

Major Depressive Disorder is a chronic and debilitating mood disorder, affecting approximately 320 million people worldwide The symptomatology of depression comprises low mood and in some cases suicidality along with anhedonia (Treadway and Zald, 2011) and cognitive symptoms. The cognitive symptoms of depression include intense rumination about past AMs, overgeneral memory recall as well as a bias towards negative memories (Connolly and Alloy, 2018, Hamlat et al., 2015). Rumination in particular has been in the centre of several cognitive models that try to explain the initiation of a depressive episode and the persistence of low mood in MDD and is also considered as a risk factor for depression (Hamlat et al., 2015, Williams et al., 2007). Several depressive symptoms, including rumination and anhedonia, are also present during remission from depression. Despite their prevalence and debilitating nature however, anhedonia and rumination are not easily targeted by commonly prescribed antidepressants.

Current common antidepressant treatments not only fail to successfully target anhedonia and rumination, but also present with certain limitations associated with their mechanism of action. A relatively long time is required for their effects to become detectable; they have a rather low response rate and approximately 1/3 of patients with MDD do not respond to current treatments (Cipriani et al., 2018). Ketamine, that has emerged, in the last two decades, as a potent antidepressant with robust effects, is regarded as a major advancement in the treatment of mood disorders (Zanos and Gould, 2018). Its fast-acting and relatively long lived antidepressant action along with the pharmacology of the drug and its metabolites, that target glutamate (ketamine, norketamine) and AMPA ((2R,6R)-HNK) receptors in the brain could not only help with the development of future treatments but could also enrich our knowledge of the biology of depression. Moreover, several research studies have underlined the positive effects of ketamine on rumination and anhedonia.

In order to understand however, how ketamine exerts its antidepressant action in the brain, we need to have a good understanding of the brain areas that are important for depression and could potentially be targeted by ketamine as an antidepressant. Several MRI studies have used resting state as well as fMRI paradigms in order to identify the brain areas that present with altered function in depression (Drevets et al., 2008a). However, most of these studies have recruited depressed individuals who were receiving antidepressant treatment at the time or shortly before the time of the scan. As a result, the findings of these studies could be obscured by the effects of antidepressant treatment in the brain. The MRI studies that have used drug naïve individuals and resting state to examine changes in brain connectivity in MDD are summarized in a table that can be found in Appendix A. Due to the relatively small number of these studies as well as the different methodologies that they apply to their data, it is rather difficult to draw robust conclusions as to the brain areas that appear to be consistently affected in drug-naïve, depressed individuals. Some of the findings from these studies point to an increased connectivity within the DMN during resting state(Zhang et al., 2011). More research is thus required in order to understand the brain areas and networks that are affected in depression.

The study of depressed individuals, however, presents not only with practical difficulties due to the confounding factor of antidepressant medication but also with several ethical issues. These issues evolve around the well-being of research participants and the effect that a potential discontinuation of their treatment might have on their mental health. Remitted depressed volunteers, on the other hand who do not present with depressive symptoms, have stable mood and are not on antidepressant treatment could prove a much easier cohort to study. Moreover, research has shown that remitted depressed individuals still experience increased anhedonia and rumination as well present with altered brain activity (Lally et al., 2015, Young et al., 2016), similar to that associated with depression. Specifically, the presence of anhedonia and increased rumination, although not clinically significant, could indicate that the brain areas and networks that underlie these processes and are affected in depression could still present with altered function in remission (Lally et al., 2015, Young et al., 2016, Young et al., 2018). As a result, these volunteers are a useful cohort to study, in a controlled, experimental setting, some of the key depressive symptoms and investigate how drugs with antidepressant actions could alter activation in brain areas important for depression.

In the present study we have used, remitted depressed volunteers who have been treatment naïve at least three months prior to taking part in the study in order to examine the effects of ketamine, 2h after the administration of the drug, with the use of fMRI. We have focused on reward processing that is related to anhedonia as well as AM recall and rumination. Our aim was to identify brain areas that present with altered activation in our cohort and are modulated by ketamine, 2h after the drug administration, when its antidepressant effects first become detectable.

These brain areas could be viewed as neuroimaging markers that would help us understand the delayed effects of ketamine's administration. In the chapter that follows, we will briefly summarize our main findings and discuss them in relation to the literature around the antidepressant actions of ketamine in the brain. Moreover, we will examine changes in brain activity produced by ketamine in association to the effects that common antidepressant medications produce in brain areas associated with Am recall and rumination as well as anhedonia. Finally, we will discuss some important considerations around this research, and potential future directions.

Anhedonia

1 Anhedonia and reward processing in remitted depressed volunteers

Anhedonia was measured in our study with the use of the SHAPS scale. This scale was administered at the start of each study days as well as 2h after the drug administration. As we expected, the baseline levels of anhedonia in our cohort of remitted depressed volunteers were very low. Ketamine, 2h after its administration did not produce any significant changes in the SHAPS scores. However, studies that have looked at anhedonia during depression and used the SHAPS reported that ketamine produces a rapid decrease in anhedonia, as early as 40min after the drug administration and that these anti-anhedonic effects could last up to three days after a single ketamine infusion without additional antidepressant treatment (Lally et al 2015).

The absence of a significant effect of ketamine on the SHAPS scores in our cohort could be attribute to either the recovery of our participants from all depressive symptoms including anhedonia or could be due to the reduced sensitivity of the SHAPS. The SHAPS is specifically designed to capture anhedonia during the depressed state (Snaith et al., 1995). As a result, the scale might not be sufficiently sensitive to depict the very low levels of anhedonia that could be present in our group as well as capture any subtle changes that ketamine might produce 2h after its administration. Examination of the available anhedonia questionnaires however, failed to provide us with a more sensitive and equally well-validated measure.

The neuronal correlates of ketamine's effects on anhedonia have mainly been examined using PET, in order to capture the changes in glucose metabolism that could underlie the anti-anhedonic action of ketamine. Specifically, PET studies have shown that ketamine, 2h after its administration produced a significant increase in glucose metabolism in the dACC and the putamen but not the VS (Carlson et al., 2013). Moreover, the baseline glucose metabolism of the dACC under placebo, was correlated with the improvement of depressive symptoms under ketamine. The dACC and the putamen are areas that are involved in reward processing, learning and decision making and have a known role in mood disorders. The increase in glutamate metabolism that ketamine produces, 2h after its administration, in these brain areas was associated with increased motivation towards or ability to anticipate pleasurable experiences (Carlson et al., 2013).

1.1 The mid task

Our study has used the MID task, a task that allows the examination of anticipatory and feedback-related processes while performing a monetary reward task (Knutson et al., 2000, Knutson et al., 2001). The MID was used in order to capture any effects that ketamine might have, 2h after its administration in brain areas that are important for reward anticipation and feedback. Analysis of the MID task has shown that deficits in brain activity during reward in depression are mainly associated with decreased activations in the NAc (Epstein et al., 2006, Keedwell et al., 2005). However, the decreased activation of the NAc could be an effect of antidepressant treatment since studies in drug-naïve MDD patients (Knutson et al., 2008) as well as non-depressed individuals with high anhedonia (Harvey et al., 2007) have failed to replicate this finding. A recent meta-analysis of fMRI studies that have used the MID task in depression revealed that depression is characterised by reduced striatal activity, especially during the feedback phase of the task (Keren et al., 2018).

In our study, and in the placebo group, when activity was examined in brain areas that are important for reward processing in the MID (*a prior* defined ROI analysis in the striatum, VTA, insula and amygdala), we found during the for anticipation of win trials, compared to neutral trials, remitted depressed volunteers presented with significantly decreased activations in the VS, the insula and the putamen. For the anticipation of a high win trials, compared to a low win trials, the VS and the VTA were decreased in activation. When the feedback phase of the task was analysed, we found that the VTA presented with decreased activity during unsuccessful low win trials compared to neutral ones. There is no control group in this study, but the overall pattern of decreased activity is supportive of a hypothesis of persistent deficits in reward processing during remission.

Ketamine, 2h after its administration, did not significantly alter task performance but did produce significant changes in our *a priori* defined ROIs. These changes were more prominent during the feedback phase of the MID. Specifically, ketamine increased the activation of the VS and the caudate during the feedback phase of successful low win trials and unsuccessful low win trials respectively. Activity in the VTA was also increased during the feedback phase of unsuccessful trials, irrespective of the magnitude of the lost reward.

To our knowledge, our study is the first to examine the effects of ketamine on the MID task, 2h after the drug administration, so direct comparisons of our findings with the existing literature are not possible. However, in depressed individuals ketamine induces an increase in VS metabolism (Carlson et al., 2013) that related to the decrease in anhedonia. When previous findings are combined with our results of increased activations in striatal regions, we can hypothesise that ketamine's delayed effects on reward feedback underlie a positive influence of the drug on those individuals with higher levels of anhedonia. Interestingly, the increased striatal and VTA activity after ketamine on the MID were most prominent for unsuccessful trials and/or trials associated with lower monetary rewards. This finding considered together with the meta-analysis findings which demonstrate reduced striatal activity during the feedback phase of the MID task in depression, could also point towards ketamine increasing the sensitivity to feedback. This increase was not detected for the higher reward trials, where the magnitude of the reward could be sufficient to mask deficits in motivational processing in the remitted depressed group of our study.

2 Current antidepressants and anhedonia

Current first-line treatment for depression includes tricyclic antidepressants as well as serotonin and noradrenaline reuptake inhibitors, focusing most of the research around depression on these two neurotransmitter systems. Anhedonia, however, is directly linked to dopaminergic pathways and as such the partial inability of common treatments to significantly improve this symptom is not surprising. Evidence suggests that standard medication for depression are poor in alleviating anhedonia, which is often the last symptom to be improved by SSRIs (Boyer et al., 2000, Shelton and Tomarken, 2001). There is also evidence suggesting that SSRIs might induce some aspects of anhedonia (Hindmarch, 1998, Price et al., 2009). In a review discussing the role of dopamine-targeting drugs for treatment of depression, Agyropoulos and Nutt (2013) suggest that dopamine enhancing agents could be helpful for the treatment of the anhedonia-related symptoms of depression especially in patients with very robust deficits in reward and motivation.

Several fMRI studies as well as recent meta-analysis around antidepressant effects, have shown that antidepressant treatment increases the activation in brain areas associated with reward processing, such as the caudate, the NAc, as well as the insula (Zhang et al., 2013, McCabe et al., 2010). However, some of these studies have employed emotional processing tasks that do not directly target motivational anhedonia (as the MID) so we can only hypothesize that the changes observed in these studies could also be associated with our findings and indicate the positive effect of antidepressants and ketamine in reward related processes.

Ketamine, which primarily targets glutamate receptors, increases activation in the reward circuit. Reward processing, however, is mainly mediated by dopamine. At the neural level, as far as the interplay between ketamine and dopamine is concerned, there is evidence that ketamine acts as a partial agonist of the dopamine D2 receptor and has been found to increase dopamine levels in the striatum including the caudate and the putamen (Lally et al., 2014). In addition to this direct effect, the glutamatergic system which is ketamine's primary target produces downstream effects on dopaminergic activity, and this may also be a potential route for ketamine's anti-anhedonic action. However, these studies concern the effect of ketamine during its infusion and do not speak to its delayed action. In order to demonstrate the link between the dopamine system and the delayed effects on reward processing after ketamine, a combined PET-MR study of both dopamine release and MID-related activation would be needed.

Rumination

Rumination is measured in our study with the use of the RRS which is administered on the screening day in order to provide us with a baseline measure of ruminative thinking in our cohort of remitted depressed volunteers. This scale was specifically developed to measure rumination that is not confounded by depression (Treynor et al., 2003). In our group, we found increased scores in the RRS that exceeded the average reported in the literature for the healthy population (Nolen-Hoeksema, 1997). Moreover, the reported difference in the RRS scores, between males and females, with females usually reporting higher scores than males, is not present in our group which is rather well gender-balanced(Johnson and Whisman, 2013). This finding indicates the presence of rumination in our cohort.

Ruminative thinking has mainly been examined in depression using resting state fMRI. This is not surprising since rumination has been linked to an increased self-focus and increased activation in the DMN (Lehmann et al., 2016, Sheline et al., 2009). Thus, examination of this network would be useful to understand the neuronal correlates of rumination in depression. Patients with MDD show increased resting state activity, compared to healthy controls, in several DMN regions including the PCC, which is a key node of the DMN. Whereas increased connectivity between the PCC and sgACC has also been identified in MDD during rumination (Berman et al., 2011). The increased connectivity of the DMN has been associated with higher levels of maladaptive rumination (Hamilton et al., 2015) which is linked to increased negative affect and depression.

Ruminative thoughts, in healthy as well as depressed individuals mainly evolve around past autobiographical events. In depression, overgeneral AM recall that is one of the most common cognitive characteristics of the illness (Williams et al., 2007), as well as the bias toward negative memory retrieval (Connolly and Alloy, 2018), direct ruminative thinking towards negative AMs. Studies that have used the AMT have confirmed overgeneral AM in depression (Young et al., 2014) as well as a bias towards a more detailed recollection of negative memories which also persists in remission (Young et al., 2016). The DMPFC along with temporal regions, the caudate and the amygdala have been implicated in this biased recall and present with increased activation in MDD (Koseki et al., 2013, Young et al., 2013).

3 Emotionally valenced AM recall in remitted depressed volunteers

In our study we have developed a novel task, the VAMP, which would help us examine rumination and AM recall in our cohort of remitted depressed volunteers and identify any changes that ketamine might produce in the activation of brain areas that are significantly engaged during task performance. During the VAMP task a 12 sec long window-retrieval phase-is given to participants in order to focus on and recall specific autobiographical events, with different emotional valence: positive, negative and neutral. Whole brain analysis of the task revealed that in the placebo group and during active recall compared to the control condition, the task alters brain activation in areas that are important for successful memory retrieval and the visual imagery associated with the retrieved memory, namely the precuneus, the middle frontal gyrus and the thalamus.

A task-based connectivity analysis was also performed for the retrieval phase of the task and the aim of this analysis was to examine the connectivity between seed regions that are important for rumination and AM retrieval, namely the amygdala, the PCC and the sgACC, with the rest of the brain during recall of emotionally valenced AMs. The connectivity of the amygdala with the rest of the brain was significantly different between the VAMP contrasts and modulated by ketamine.

Specifically, for the placebo session, increased connectivity was identified between the amygdala and the visual cortex as well as the amygdala and the hippocampus when recall of positive and negative memories was, separately, compared to neutral memories. Moreover, when positive AM retrieval was compared to negative, connectivity between the amygdala and the visual cortex increased for positive AMs, compared to neutral. The connectivity between the amygdala and the hippocampus decreased for the same contrast. These findings could indicate the increased visual imagery associated with positive memory recall in our cohort as well as the reduced emotional regulation that might be associated with positive memory recall compared to negative, in order to successfully perform the VAMP task.

When VAMP connectivity was examined using PPI and compared between the ketamine and placebo sessions, significant changes were identified only between the connectivity of the amygdala with the rest of the brain. Ketamine, 2h after its administration, decreased the connectivity between the bilateral amygdala and the hippocampus, and this finding was present during negative AM recall compared to neutral.

Ketamine also disrupted the connectivity pattern between the amygdala and the visual cortex, Specifically, the visual cortex presented with decreased connectivity with the amygdala when positive and negative AM recall were respectively contrasted to neutral AM recall and compared between the ketamine and placebo sessions. The decreased connectivity between the amygdala and the visual cortex could be associated with reduced visual imagery during recall of those memories, after ketamine which could interfere with reconsolidation processing and consequently alter the way these memories are remembered.

4 The effects of ketamine on the VAMP task

In the literature, the delayed effects of ketamine, in recall and rumination of AMs are very poorly examined and understood. Two resting state connectivity studies in healthy volunteers showed that 24h post ketamine, DMN connectivity decreases (Lehmann et al., 2016, Scheidegger et al., 2012). Both these studies have tried to relate the delayed effects of ketamine on the DMN connectivity, with the increased rumination that is observed during depression as well as the bias towards negative affect in order to draw conclusions about the antidepressant actions of the drug. They suggest that ketamine, by reducing DMN connectivity, would have a positive effect on rumination in depression. However, the luck of replication of these findings as well as the fact that they have used healthy controls makes it very difficult to draw conclusions about the antidepressant actions of ketamine.

In depressed volunteers, to our knowledge, there are so far only three studies that have examined the delayed, 24h post administration, effects of ketamine with the use of fMRI, and compared them to healthy controls. In these studies, ketamine was administered to depressed individuals as a treatment and the main focus of these studies was to relate ketamine's change in brain activations with the changes in the depressive symptoms produced by the drug. These studies in general identified ketamine-induced increases in the brain connectivity (Abdallah et al., 2017) as well as increased BOLD responses in the sgACC (Downey et al., 2016), the insula and the caudate (Murrough et al., 2015). These changes were positively correlated with the improvement of depressive symptoms produced by the drug. Most importantly, Murrough and colleagues used a facial emotion-perception task and showed that ketamine enhanced neuronal responses to positive emotion in the right caudate in depressed patients, compared to placebo.

Our findings in remitted depressed volunteers and 2h after ketamine administration, show that the drug, disrupts the connectivity between the amygdala and the visual cortex as well as the hippocampus during emotional memory recall. This disruption in the connectivity could be linked to cognitive processes that include memory retrieval and reconsolidation as well as emotional regulation. Taken together, our findings along with ketamine's delayed effects on depressed as well as healthy volunteers point towards the drug targeting cognitive processes such as rumination and memory recall with a specific effect on the emotional components that underlie these processes.

These findings become particularly interesting when viewed in association with the cognitive models that have been proposed in the literature and try to explain the initiation and prolongation of depressive episodes. According to these models altered emotional recall as well as a bias towards negative memories and brooding (maladaptive rumination) are the cognitive processes that are mainly affected in depression(Connolly and Alloy, 2018, Thomsen, 2006). In summary, these models propose that bias towards negative memory recall, along with increased rumination about those negative AMs and the reduced positive affect that is associated with depression create a cognitive "vicious" circle that is very hard to break and could even persist in remission (Beck and Bredemeier, 2016, Williams, 2006). The delayed effects of ketamine in decreasing resting state connectivity which could be positive for rumination and the increased responses to positive emotions along with alterations in memory retrieval and reconsolidation processes could have positive effects on the cognitive processes that are affected in depression.

These findings, however, arise from studies that have used different samples (depressed, remitted depressed and healthy controls), have looked at different time points during the ketamine infusion (2h as well as 24h) and used different MRI paradigms. More research is needed that uses consistent paradigms among studies in all of these groups to provide us with a broader understanding of ketamine's delayed effects in the brain. The drug effects should also be examined at different time points during ketamine's antidepressant action in order to determine whether and how ketamine's delayed effects change overtime. Moreover, it would be useful to study ketamine in relation to other antidepressant medication, with similar and different mechanisms of action in order to examine whether ketamine's effects could be linked to those of other antidepressant treatments.

5 Current antidepressants and rumination

Research around the effects of current antidepressant treatment on rumination is rather limited. Most of the fMRI studies focus on the effects of antidepressant medication on emotional processing and have shown that that the affective network and mainly the amygdalae are a common target of current antidepressant treatment (Wessa and Lois, 2015). These effects seem to be more prominent in emotional tasks.

Specifically, it has been shown that the increased reactivity of the amygdala observed in depressed patients during an emotional-processing task normalises after treatment with SSRIs. This normalization occurred after 8 weeks of antidepressant medication treatment (Sheline et al., 2001) and since increased amygdala reactivity is associated with a bias towards negative memories, it was suggested that this reduction could help with maladaptive rumination. In another study that investigated the effects of fluoxetine (8 weeks) during processing of sad faces, it was shown that in MDD, amygdala activity is positively correlated with medial temporal and ventral occipital regions and anticorrelated with the ACC (Tao et al., 2012). Moreover, antidepressant medication significantly increased the coupling of the amygdala with frontal regions (lateral PFC), the thalamus and the striatum and this result could indicate the role of antidepressant medication in altering the interplay between emotional reactivity, mediated by the amygdala, and cognitive control mainly associated with frontal region thus leading to a normalisation of the heightened emotional responses observed in depression(Chen et al., 2006) (Chen et al., 2008).

Ketamine's delayed effects also alter amygdala activity and this has been linked to improved emotional processing in both healthy as well as depressed individuals (Murrough et al., 2015, Scheidegger et al., 2012). As result the amygdala could be a prominent target for the mechanisms of action of antidepressant medication including ketamine.

Considerations and limitations

The findings of our study in general, indicate that ketamine, 2h after its administration, produces significant changes in the activation of brain areas that present with altered function in depression. These brain areas also play a key role in mediating anhedonia and emotional memory recall that could be linked to rumination. Most of the significant changes in brain activity that we identified, 2h after the ketamine administration and in our cohort, were increases. This could lead to the conclusion that ketamine would produce a general increase in brain activity, 2h after its administration, that is not specifically related to the antidepressant actions of the drug. However, the increases that we observed in our data set, occurred under task contrasts specifically designed to explore those aspects of emotionally valenced AM recall and anhedonia that are associated with depression. Moreover, the fact that ketamine's significant changes in brain activations were identified only when our *a priori* ROIs were examined and not at a whole brain level, further indicates that ketamine's effects were targeted to specific neuronal processes. Finally, ketamine also produced decreases in the activation of several ROIs during the MID task contrasts, however, most of these decreases did not survive correction for multiple comparisons.

In our study, a single dose of ketamine, assessed 2h after its administration, did not produce any significant changes in anhedonia as measured by the SHAPS and the SHAPS scores did not correlate with the changes in brain activity that ketamine produced. Additionally, during the VAMP task, emotional valence of the positive and negative AMs did not change after ketamine, and no correlations were identified between the effects of ketamine on brain activation and those ratings. Since in our cohort, ketamine did not produce any significant behavioural changes that could relate to the drug's antidepressant actions, we would refrain from calling the effects that ketamine produced in the brain as antidepressant. This, however, does not diminish the importance of those effects which in depressed volunteers could indeed be associated with a marked improvement in depressive symptoms.

One major consideration in our study as well as generally in research using ketamine, is the lack of an effective placebo. In our study the dissociative and psychotomimetic effects of ketamine were very pronounced and not only jeopardised the design of the study but also caused a significant burden in the well-being of our volunteers during and around the time of the infusion. The unpleasantness of the dissociative and psychotomimetic effects as well as the physical symptoms that ketamine administration produces, were captured in our study as increased anhedonia during the infusion (PSI anhedonia sub-scale) as well as reduction in the SNK-W scores for well-being.

We anticipated that ketamine would produce robust side-effects that could potentially affect the integrity of data collection since both the investigators as well as the volunteers were able to, most of the time, accurately distinguish the ketamine from the placebo session. For that reason, we recruited sufficient remitted depressed volunteers, in order to be able to detect a potential effect of session by separately analysing the sessions were ketamine was administered first and comparing them to the placebo sessions. No effects of the drug administration order were identified in the behavioural as well as the fMRI data from this study. While the unblinding of ketamine could not be avoided in this study a number of steps were taken to minimise its impact. First, the drug was administered blind to the researcher conducting all the testing. Second, the main outcome measures (VAMP and MID) were fully automated tasks in the scanner. Third, with the testing occurring distant from the infusion it was ensured that all dissociative and psychotomimetic effects of ketamine had passed.

Future directions

In our study the brain areas that appear to be particularly sensitive to the effects of ketamine, 2h after the drug administration include the amygdala and hippocampus, during emotionally valenced recall of AMs as well as the VS and VTA during the feedback phase of the MID. These areas could thus serve as potential neuroimaging markers in order to examine the delayed effects of ketamine and how these effects are associated with the antidepressant actions of the drug. Future studies of these brain areas in remitted depressed individuals could answer questions around the neural processes that are mediated by these regions and what is their potential role in cognitive processing that could also be affected in depression. In depressed volunteers the study of these areas would allow for a more direct link between the antidepressant effects of ketamine and the changes in the brain activity that are produced by the drug. Moreover, these areas could be useful in the study of other compounds that target the glutamatergic system and could have antidepressant action.

In the VAMP task, the delayed effects of ketamine during the recall of positive and negative memories, compared to neutral, ware associated with disruption in the connectivity of the amygdala with the visual cortex as well as the amygdala and the hippocampus. The decreased connectivity between these areas during emotionally valenced AM recall, which could be associated to emotional memory recall and reconsolidation processes, could indicate that ketamine might be particularly helpful in depression when combined with psychotherapy of cognitive behaviour therapy. Several therapeutic techniques, including MEST (Memory Specific Training) as well as Mindfulness based training , try to reverse the cognitive bias towards negative memory recall that is observed in depression by inducing negative AM recall and subsequent re-consolidation of these memories in a controlled environment(Kohler et al., 2015). Ketamine's delayed positive effects in the neural processes that might mediate the reconsolidation of those memories might be might particularly beneficial for these therapeutic strategies of depression.

More research is required in remitted depressed volunteers as well as depressed individuals in order to better understand how ketamine manipulates these processes. The delayed effects of ketamine at multiple time points during the window of its antidepressant action could be examined in order to detect the best point in time for the induction of AM recall that would coincide with the peak of ketamine's antidepressant effects on those memory processes. Additionally, if ketamine, as an antidepressant could interfere with memory recall and reconsolidation, special attention should be given during and after its administration since exposure of individuals to stressful or traumatic events during the therapeutic window of the drug, could potentially be harmful, especially for depressed volunteers.

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Appendix A

CONNECTIVITY ANALYSIS IN TREATMENT NAÏVE, MDD PATIENS						
Seed Based Functional Connectivity						
Study	**Subjects**	**Methodology**	**Summary of Results**			
Yang et al., 2017	first episode MDD patients, n=40* (21 males) HC, n=36** (17 males) *3 participants were excluded **4 participants were excluded	Connectivity Analysis The seeds selected for the FC analysis included • Bilateral VC (Ventral Caudate) • STG (Superior Temporal Gyrus) The time course of these seed regions was correlated with the entire brain	**MDD > HC**			
				VC	Right	R Occipital Lobe, Cuneus
					Left	R Occipital Lobe, Cuneus
					Left	L Parietal Lobe, Precuneus
				STG	Left	R Angular Gyrus
					Left	L Occipital Lobe, Cuneus
			MDD< HC			
					Right	R Middle Temporal Gyrus
				VC	Left	R Superior Parietal Lobule
					Left	R Superior Frontal Gyrus
Gong et al., 2017	First episode MDD patients, n=80 (33 males) HC, n=43 (23 males) *5participants were excluded **1 participant was excluded	Connectivity Analysis The left and right NAc (Nucleus Accumbens) ware used as seeds and examine separately. The time course of the seed regions was correlated with the rest of the brain	**MDD < HC**			
						Bilateral Caudate
						Medial Orbital Frontal Cortex
				NAc	Left	Middle Orbital Frontal Cortex
						Rostral ACC
						Left Superior Temporal Gyrus
						Insular Lobe
						Left Superior Temporal Gyrus
						Insular Lobe
				NAc	Right	Bilateral Middle Orbital Frontal Cortex
						Right ACC
						Left Medial Orbital Cortex
			MDD>HC			
				NAc	Right	Bilateral Dorsal Medial Prefrontal Cortex
						Left Dorsal ACC

Guo et al., 2015a	MDD patients, n=44 (22males) HC, n=44 (20males)	Connectivity Analysis The time course of the Bilateral Crus I was correlated with the rest of the brain	**MDD> HC** Right — R Inferior Frontal Cortex Crus I — Right — R Superior Temporal Pole Right — Bilateral MPFC Right — L Middle Temporal Gyrus
Guo et al., 2015b	MDD patients, n=44 (22 males) HC, n=44 (20 males)	Connectivity Analysis The time course of the Bilateral insular cortex seeds was correlated with the rest of the brain	**MDD<HC** Right — L Middle Frontal Gyrus L Superior Temporal Gyrus Insula — R Putamen R Middle Occipital Gyrus Left — L Superior Temporal Pole R Middle Occipital Gyrus
Cao et al., 2012	MDD patients, n=42 (18 males) HC, n=32 (17 males)	Connectivity Analysis The time course of the Bilateral hippocampal seeds was corrected with the rest of the brain	**MDD>HC** Bilateral Middle Frontal Gyrus Left Hippocampus R Inferior Parietal Lobule Right — R Cerebellar Tonsil
ICA (Independent Component Analysis)			
Study	**Subjects**	**RSNs (Resting State Networks)**	**Summary of Results**
Zhu et al., 2012	MDD patients, n=37* (17 males) HC, n=37** * 5 participants were excluded **4 participants were excluded	Connectivity Analysis ICA was conducted and a template of the DMN was used to examine connectivity changes between the two groups	Within the DMN **MDD> HC** • Dorsal MPFC/Ventral ACC, • Ventral MPFC • Medial Orbital PFC **MDD<HC** • PCC/Precuneus, • Right AG, • Left AG/Precuneus

Veer et al., 2010	MDD patients, n=23* (8 males) HC, n=19 (8 males) *4 participants were excluded	<u>Connectivity Analysis</u> The following ICA networks were identified and compared between the two groups 1. Primary Visual Network 2. Lateral Visual Network 3. Medial Visual Network 4. Sensory - Motor Network 5. Right Lateral Network 6. Left Lateral Network 7. Precuneus 8. Ventral Stream Network 9. Medial Temporal Network 10. Salience Network 11. Task Positive Network 12. Auditory Network 13. DMN	<u>Within the Medial Visual Network (RSN3)</u> **MDD<HC** • The Lingual Gyrus with the rest of the network <u>Within the Auditory Network (RSN12)</u> **MDD<HC** • Amygdala and Left Insula with the rest of the network • Right Superior Temporal Gyrus **MDD>HC (within the network)** • Right Inferior Frontal Gyrus <u>Between the Task Positive Network (RSN11) and the rest of the brain</u> **MDD<HC** • The Left Frontal Pole with the rest of the network

ROI- to -ROI			
Study	**Subjects**	**ROIs**	**Summary of Results**
Zhu et al., 2017	first episode MDD patients, n=31 (14 males) HC, n=32 (15males)	Connectivity Analysis The following ROIs of the DMN were defined and included 1. Anterior MPFC 2. PCC 3. Dorsal MPFC 4. TPJ (Temporo-parietal junction 5. LTC (Lateral Temporal Cortex) 6. TempP (Temporal Pole) 7. Ventral MPFC 8. Rsp (Retrosplenial cortex) 9. pIPL (posterior Inferior Parietal Lobule) 10. PHC (Parahippocampal Cortex) 11. HF (Hippocampal Formation) The three DMN subsystems were defined as 1. Midline core: anterior MPFC and PCC 2. dMPFC subsystem: dMPFC, TPJ, LTC and TempP 3. MTL subsystem: vMPFC, pIPL, Rsp, PHC, HF	Between the DMN ROIs Within system connectivity in the dMPFC subsystem **MDD>HC** • dMPFC-Temp • TPJ-LTC Inter system connectivity between the dMPFC and MTL subsystems **MDD>HC** • TPJ-PHC (inter-system connectivity between the dMPFC and MTL subsystems) • LTC-PHC(inter-system connectivity between the dMPFC and MTL subsystems) • TempP-vMPFC(inter-system connectivity between the dMPFC and MTL subsystems) • TempP-pIPL(inter-system connectivity between the dMPFC and MTL subsystems) • TempP-Rsp(inter-system connectivity between the dMPFC and MTL subsystems) • TempP-PHC(inter-system connectivity between the dMPFC and MTL subsystems) Within the DMN subsystems **MDD>HC** • dMPFC subsystem Between the DMN subsystems **MDD>HC** • dMPFC subsystem - MTL subsystem
Wei et al., 2017	first episode MDD patients, n=49 (17 males) HC, n=50 (17 males)	Connectivity Analysis Correlation analysis between the bilateral amygdala ROI and bilateral PFC mask including Brodmann area 9-12, 24, 25, 32 and 44-47	**MDD<HC** • amygdala - VPFC • amygdala - DLPFC

Tao et al., 2011	first episode MDD patients, n=15 (8 males) HC, n=37 (8 males)	Connectivity Analysis The whole brain was parcellated in 90 ROIs based on the anatomical labelling atlas and the time series of these ROIs were compared between the two groups	**MDD>HC** • Left Hippocampus - Right Parahippocampal Gyrus • Right Inferior Frontal Gyrus - Right Inferior Orbitofrontal Cortex • Right Medial Frontal Gyrus - Right Inferior Frontal Gyrus • Right Cuneus - Left Superior Occipital Gyrus • Right Superior Orbitofrontal Cortex - Right Inferior Orbitofrontal Cortex **MDD<HC** • Bilateral Insula - Bilateral Putamen • Left Superior Frontal Gyrus - Right Insula • Left Precentral Gyrus - Left Inferior Frontal Gyrus • Right Inferior Frontal Gyrus -Right Supramarginal Gyrus • Left Precentral Gyrus - Left Inferior Parietal Lobule • Right Lingual Gyrus - Right Fusiform Gyrus • Right Angular Gyrus - Right Precuneus • Left Superior Orbitofrontal Cortex – Left Inferior Orbitofrontal Cortex
OTHER CONNECTIVITY TECHNIQUES			
Study	**Subjects**	**ROIs**	**Summary of Results**
Wang et al., 2012	first episode MDD patients, n=18 (9 males) HC, n=18 (9 males)	Connectivity Analysis ALFF (Amplitude of Low Frequency Flactuations) and fALFF (fractional ALFF) analysis was performed and differences between the two groups were explored	ALFF Results **MDD>HC** • Right Fusiform Gyrus • Right Anterior Lobe of the Cerebellum • Right Posterior Lobe of the Cerebellum **MDD<HC** • Left Inferior Temporal Gyrus • Bilateral Inferior Parietal Lobule • Right Lingual Gyrus fALFF Results **MDD>HC** • Right Precentral Gyrus • Bilateral Fusiform Gyrus • Bilateral Anterior Lobes of the Cerebellum • Bilateral Posterior Lobes of the Cerebellum **MDD<HC** • Left Dorsolateral Prefrontal Cortex • Bilateral Medial Orbitofrontal Cortex • Bilateral Middle Temporal Gyrus • Left Inferior Temporal Gyrus

			• Right Inferior Parietal Lobule
Zhang et al., 2011	first episode MDD patients, n=31* (8 males) HC, n=64**(30 males) * 1 participant was excluded ** 1 participant was excluded	<u>Connectivity Analysis</u> Graph connectivity theory trying to examine the topological organisation of functional brain networks in MDD and compare them to HC	**MDD** • decreased path length • increased global efficiency implying a disturbance of the normal global integration of whole-brain networks Increased nodal centralities were observed in • the Caudate Nucleus • the DMN DEcreased nodal centralities were observed in • the Temporal Lobes • the Occipital Lobes

Appendix B

K.A.R.M.A _LEDS

Adjustment of the LEDS interview for the identification of Positive, Negative and Neutral Life Events and Difficulties.

Introduction

Now I would like to ask you about last year, and by that I mean from ... since … and perhaps a bit further back.

We will be focusing on the positive and negative things that may have happened to you or to people close to you during that time.

So, I will need to find out a little bit about those people

By people close to you I mean: *(ask each next questions with an interrogative tone of voice, as if to say "Do you have one")*

Husband /wife or boyfriend/girlfriend?

Children?

Brothers/Sisters?

Parents? /Step-parents?

Other household members?

Close friends or Confidants?

At the end of this interview, I would also like you to describe one of your daily routines that feels neutral to you.

For example, a day at work/university/home that was not particularly exciting or sad.

Positive Event

First of all lets start with what you think was the most positive thing that happened to you during the last 12 months.

Make sure to find out:

a. *When exactly did it happen?*
b. *Was someone else involved?*
c. *How did you feel about it then and how did you feel about it a couple of weeks after?*

Event keywords for statements:

1.	11.
2.	12.
3.	13.
4.	14.
5.	15.
6.	16.
7.	17.
8.	18.
9.	19.
10.	20.

During the same year, did anything else happen that was also very positive/exciting?

Make sure to find out:

a. *When exactly did it happen?*
b. *Was someone else involved?*
c. *How did you feel about it then and how did you feel about it a couple of weeks after?*

Event keywords for statements:

1.	11.
2.	12.
3.	13.
4.	14.
5.	15.
6.	16.
7.	17.
8.	18.
9.	19.
10.	20.

If nothing else positive happened during the last year or the participant can recall only one positive event then say:

How about going a bit further back in time? Let's say the year before that.

Did anything really positive happen from … since …

Make sure to find out:

a. *When exactly did it happen?*
b. *Was someone else involved?*
c. *How did you feel about it then and how did you feel about it a couple of weeks after?*

Event keywords for statements:

1.	11.
2.	12.
3.	13.
4.	14.
5.	15.
6.	16.
7.	17.
8.	18.
9.	19.
10.	20.

If two or more events are being mentioned then ask the participant.

Do you feel that these events were equally positive or did one of them make you feel more happy?

Which one?

And how do you feel thinking about this event now?

Negative Event

And how about the most negative and sad experience that you had during the last year?

Make sure to find out:

a. *When exactly did it happen?*
b. *Was someone else involved?*
c. *How did you feel about it then and how did you feel about it a couple of weeks after?*

Event:

1.

2.

3.

4.

5.

6.

7.

8.

9.

10.

11.

12.

13.

14.

15.

16.

17.

18.

19.

20.

Did anything else happen that was also very sad?

Make sure to find out:

a. *When exactly did it happen?*
b. *Was someone else involved?*
c. *How did you feel about it then and how did you feel about it a couple of weeks after?*

Event:

1.

2.

3.

4.

5.

6.

7.

8.

9.

10.

11.

12.

13.

14.

15.

16.

17.

18.

19.

20.

If nothing else negative happened during the last year or the participant can recall only one negative event then say:

How about going a bit further back in time? Let's say the year before that.

Did anything negative happen from … since …?

Make sure to find out:

a. *When exactly did it happen?*
b. *Was someone else involved?*
c. *How did you feel about it then and how did you feel about it a couple of weeks after?*

Event:

1.	11.
2.	12.
3.	13.
4.	14.
5.	15.
6.	16.
7.	17.
8.	18.
9.	19.
10.	20.

If two or more events are being mentioned then ask the participant.

Do you feel that these events were equally bad/negative or did one of them make you feel worse?

And how do you feel thinking about this event now?

Neutral events

Now, as I have mentioned at the beginning, I would like you to describe one of your daily routines.

This question could be adjusted according to participants… For example, if the volunteer is a university student you could ask them to describe a normal day at the university.

Say for example:

How about a usual day at uni? Is that day associated with something particularly positive or negative? Does it make you feel sad or happy thinking about it?

Make sure to find out:

a. *When exactly did it happen?*
b. *Was someone else involved?*
c. *How did you feel about it then and how did you feel about it a couple of weeks after?*

Event:

1.	11.
2.	12.
3.	13.
4.	14.
5.	15.
6.	16.
7.	17.
8.	18.
9.	19.
10.	20.

If the participant says that they have particularly strong feeling about that routine or they associate it with something very sad. For example, hate waking up very early in the morning, have a class with a professor that they particularly dislike or learning that something very sad or happy happened while they were at uni… ask them for another daily routine.

Make sure to find out:

a. *When exactly did it happen?*
b. *Was someone else involved?*
c. *How did you feel about it then and how did you feel about it a couple of weeks after?*

Event:

1.	11.
2.	12.
3.	13.
4.	14.
5.	15.
6.	16.
7.	17.
8.	18.
9.	19.
10.	20.

When trying to obtain contextual information about the negative events make sure you cover all the probes from each appropriate section:

HEALTH

A. FOR ANY KEY ILLNESS EVENT/DIFFICULTY, SOME SUGGESTED 'PROBES':

FROM DOCTORS:

Reasons for llness.

Chances recovery/outlook.

Treatability.

Future health; implications for work.

Has anyone else had it in the family?

Lack of information from doctor.

Shortcomings in care.

IMPACT ON:

Employment; chance of losing job.

Sick pay; problems obtaining suitable care.

Manifestations.

Handicap. How needed to cut down?

Pain, symptoms.

How long in bed?

Interference with everyday life/hobbies/ future plans.

Had before? Outcome.

ILLNESS OF OTHERS ONLY:

Was it expected?

How involved were you?

Nursing; infectiousness.

Worry about dying.

Worry handicap.

Diet; incontinence; lifting.

Change behaviour/personality e.g. anger, irritability, ingratitude, blame?

Stigma/embarrassment?

ROLE CHANGES

B. FOR ANY INTERACTION CHANGE EVENT:

Temporary. How long away.

How often seen before the change?

How much did you do together?

How often do you see now? Distance.

Telephone contact.

How did you get on? How about now?

Preparation. Evidence rejection/guilt.

INCREASE IN INTERACTION:

How fitted in - space/tension.

C. FOR ANY MARRIAGE/ENGAGEMENT INVOLVING S:

How long known.

Complications/'delaying tactics'/rejections.

Family reactions.

Was there anything about him made you uneasy?

D. FOR ANY DIVORCE/SEPARATION INVOLVING S:

Reasons.

Preparation; anticipation.

Who left? What circumstances.

Forced to leave.

Anyone else involved.

'Alternative' relationship by either spouse.

Finance/housing.

Custody.

Children - their reactions etc.

Clean break/pestering/violence.

Family's reaction.

Legal advice. When.

Maintenance arrangements.

Often seen now.

LEISURE AND INTERACTION

Have you made any new friends, of either sex, at all?

Have you lost someone you were close to - either because they've moved a way, or died, or just drifted apart?

Have there been any big changes in the amount you see of your friends or relatives?

Have there been just the ... of you at home during the time.... since ...

Has anyone left the household at all?

Is there anyone you see much less of?

HOUSING

E. FOR ANY RESIDENCE CHANGE EVENT, PROBE:

Why did you move? What happened?

Decision to move.

Were there any difficulties?

Have there been any since then?

Expense.

Consequences.

Did you feel cut off? Baby-minders etc.

New friends.

Impact on job.

Problems re house/neighbours etc.

EMPLOYMENT AND SCHOOL

F. IF ANY IMPORTANT CHANGE ESTABLISHED, FIND OUT:

How came about, whose decision.

Financial implications.

Convenience, hours etc.

IF FOR S:

Travel, babysitting/ arrangements for children.

Responsibility/demandingness.

Interest; importance.

Plans for future.

CRISES

G. FOR ANY COURT APPEARANCE EVENT:

Nature of offence.

First time done it.

First time in court.

Other convictions.

Verdict. Sentence.

Financial implications.

What have other people said?

What have they said at work?

Driving affected (if licence lost etc).

Implications re other people involved.

Were you afraid they would try to get

their own back?

H. FOR ANY BURGLARY OR LOSS OR DAMAGE TO PROPERTY:

How did it come about? (S's 'fault'?)

Did you see the burglar?

How much was taken?

Problems with insurance?

Anything irreplaceable.

House damaged.

Appendix C

Rumination Scale

People think and do many different things when they feel depressed. Please read each of the items below and indicate whether you almost never, sometimes, often, or almost always think or do each one when you feel down, sad, or depressed. Please indicate what you *generally* do, not what you think you should do.

1. *almost never* **2. *sometimes*** **3. *often*** **4. *almost always***

1. Think about how alone you feel	1. ☐ 2. ☐ 3. ☐ 4. ☐
2. Think: "I won't be able to do my job if I don't snap out of this"	1. ☐ 2. ☐ 3. ☐ 4. ☐
3. Think about your feelings of fatigue and achiness	1. ☐ 2. ☐ 3. ☐ 4. ☐
4. Think about how hard it is to concentrate	1. ☐ 2. ☐ 3. ☐ 4. ☐
5. Think: "What am I doing to deserve this?"	1. ☐ 2. ☐ 3. ☐ 4. ☐
6. Think about how passive and unmotivated you feel	1. ☐ 2. ☐ 3. ☐ 4. ☐
7. Analyze recent events to try to understand why you are depressed	1. ☐ 2. ☐ 3. ☐ 4. ☐
8. Think about how you don't seem to feel anything anymore	1. ☐ 2. ☐ 3. ☐ 4. ☐
9. Think: "Why can't I get going?"	1. ☐ 2. ☐ 3. ☐ 4. ☐
10. Think: "Why do I always react this way?"	1. ☐ 2. ☐ 3. ☐ 4. ☐
11. Go away by yourself and think about why you feel this way	1. ☐ 2. ☐ 3. ☐ 4. ☐
12. Write down what you are thinking about and analyze it	1. ☐ 2. ☐ 3. ☐ 4. ☐
13. Think about a recent situation, wishing it had gone better	1. ☐ 2. ☐ 3. ☐ 4. ☐
14. Think: "I won't be able to concentrate if I keep feeling this way."	1. ☐ 2. ☐ 3. ☐ 4. ☐
15. Think: "Why do I have problems other people don't have?"	1. ☐ 2. ☐ 3. ☐ 4. ☐
16. Think: "Why can't I handle things better?	1. ☐ 2. ☐ 3. ☐ 4. ☐
17. Think about how sad you feel	1. ☐ 2. ☐ 3. ☐ 4. ☐
18. Think about all your shortcomings, failings, faults and mistakes	1. ☐ 2. ☐ 3. ☐ 4. ☐
19. Think about how you don't feel up to doing anything	1. ☐ 2. ☐ 3. ☐ 4. ☐
20. Analyze your personality to try to understand why you are depressed	1. ☐ 2. ☐ 3. ☐ 4. ☐
21. Go someplace alone to think about your feelings	1. ☐ 2. ☐ 3. ☐ 4. ☐
22. Think about how angry you are with yourself	1. ☐ 2. ☐ 3. ☐ 4. ☐

Appendix D

PSYCHOTOMIMETIC STATES INVENTORY

Please complete the following questions by circling the number that best describes your experience at the moment

	Not at all	*Slightly*	*Moderately*	*Strongly*
1. You enjoy mixing with people	3	2	1	0
2. You hesitate even when you know what you are going to say	0	1	2	3
3. Your mood is going up and down a lot	0	1	2	3
4. You feel that you can predict what is about to happen	0	1	2	3
5. You feel more sensitive to light or the colour or brightness of things	0	1	2	3
6. You feel close to people	3	2	1	0
7. You think you are being talked about	0	1	2	3
8. It is more difficult than normal to follow conversations with people	0	1	2	3
9. You feel rather indifferent about things	0	1	2	3
10. Your mind jumps a lot from one thing to another	0	1	2	3
11. You think people are saying or doing things to annoy you	0	1	2	3
12. You think other people can read your mind	0	1	2	3
13. You find it more difficult than usual to start doing things	0	1	2	3
14. You are bothered by the idea that people are watching you	0	1	2	3
15. You find activities less enjoyable than usual	0	1	2	3
16. Your mind is so full of ideas that you can't concentrate on one thing	0	1	2	3
17. You feel that people have it in for you	0	1	2	3
18. It is fun to do things with other people	3	2	1	0
19. You feel that you have special or magical powers	0	1	2	3
20. Your sense of smell is unusually strong or different	0	1	2	3
21. You want to be the centre of attention more than usual	0	1	2	3
22. Your experience of time is unnaturally fast or slow	0	1	2	3
23. You feel that no one understands you	0	1	2	3
24. You feel rather uninvolved with other people	0	1	2	3
25. People can put thoughts into your mind	0	1	2	3
26. You are experiencing something very special or important	0	1	2	3
27. Your hearing has become very sensitive	0	1	2	3
28. You find it difficult to think clearly	0	1	2	3
29. You stop to think things over before doing them	3	2	1	0
30. Your speech is difficult to understand because your words are all mixed up	0	1	2	3
31. You feel that you might cause something to happen just by thinking about it	0	1	2	3
32. You feel as though your head, limbs or body have somehow changed	0	1	2	3
33. You feel that you deserved to be punished in some way	0	1	2	3
34. When you try to concentrate many unrelated thoughts pop into your mind	0	1	2	3
35. Your thoughts are sometimes so strong that you can almost hear them	0	1	2	3
36. You have seen a person's face in front of you when no one was in fact there	0	1	2	3
37. Your thoughts stop suddenly, interrupting what you are saying	0	1	2	3
38. You have a vague sense of danger or sudden dread for reasons you don't understand	0	1	2	3
39. You would feel uncomfortable if your friends were to touch you	0	1	2	3
40. You feel that you can read other people's minds	0	1	2	3
41. Ideas and insights come to you so fast that you can't express them all	0	1	2	3
42. You think people are laughing about you behind your back	0	1	2	3
43. You have the feeling of gaining or losing energy when people look at or touch you	0	1	2	3
44. You can sense an evil presence around you, even though you cannot see it	0	1	2	3
45. You can see shapes and forms even though they aren't there	0	1	2	3
46. You are easily distracted when doing something or talking to someone	0	1	2	3
47. You are confused by too much happening at the same time	0	1	2	3
48. You believe you are a special person with an important mission	0	1	2	3

Appendix E

SHAPS

Snaith-Hamilton Pleasure Scale

This questionnaire is designed to measure your ability to experience **pleasure in the last few days**. It is important to read each statement **very carefully**.

Tick **one** of the boxes [ ] to indicate how much you agree or disagree with each statement.

1. I would enjoy my favourite television or radio programme:

   Strongly disagree [ ]
   Disagree [ ]
   Agree [ ]
   Strongly agree [ ]

2. I would enjoy being with my family or close friends:

   Definitely agree [ ]
   Agree [ ]
   Disagree [ ]
   Strongly disagree [ ]

3. I would find pleasure in my hobbies and pastimes:

   Strongly disagree [ ]
   Disagree [ ]
   Agree [ ]
   Strongly agree [ ]

4. I would be able to enjoy my favourite meal:

   Definitely agree [ ]
   Agree [ ]
   Disagree [ ]
   Strongly disagree [ ]

5. I would enjoy a warm bath or refreshing shower:

   Definitely agree [ ]
   Agree [ ]

Disagree [ ]
Strongly disagree [ ]

6. I would find pleasure in the scent of flowers or the smell of a fresh sea breeze or freshly baked bread:

   Strongly disagree [ ]
   Disagree [ ]
   Agree [ ]
   Strongly agree [ ]

7. I would enjoy seeing other people's smiling face:

   Definitely agree [ ]
   Agree [ ]
   Disagree [ ]
   Strongly disagree [ ]

8. I would enjoy looking smart when I have made an effort with my appearance:

   Strongly disagree [ ]
   Disagree [ ]
   Agree [ ]
   Strongly agree [ ]

9. I would enjoy reading a book, magazine or newspaper:

   Definitely agree [ ]
   Agree [ ]
   Disagree [ ]
   Strongly disagree [ ]

10. I would enjoy a cup of tea or coffee or my favourite drink:

    Strongly disagree [ ]
    Disagree [ ]
    Agree [ ]
    Strongly agree [ ]

11. I would find pleasure in small things, e.g. bright sunny day, a telephone call from a friend

Strongly disagree [ ]
Disagree [ ]
Agree [ ]
Strongly agree [ ]

12. I would be able to enjoy a beautiful landscape:

Definitely agree [ ]
Agree [ ]
Disagree [ ]
Strongly disagree [ ]

13. I would get pleasure from helping others:

Strongly disagree [ ]
Disagree [ ]
Agree [ ]
Strongly agree [ ]

14. I would feel pleasure when I receive praise from other people:

Definitely agree [ ]
Agree [ ]
Disagree [ ]
Strongly disagree [ ]

Appendix F

Subjective Well-being Scale Post Infusion

	Not at all	A little	Somewhat	Noticeable	Much	Very Much
1. I feel powerless and not in control of myself						
2. I feel very comfortable with my body						
3. I find it easy to think						
4. I have no hope for the future						
5. My body feels familiar						
6. I am very shy about getting to know people						
7. I am imaginative and full of ideas						
8. My environment seems friendly and familiar to me						
9. I feel weak and exhausted						
10. My emotions and sensations are dull. Nothing matters to me.						
11. My thinking is difficult and slow						
12. My feelings and behaviour are inappropriate to situations. I get upset over small things, important ones hardly affect me						
13. I find it easy to keep in touch with people around me						
14. I perceive my environment as being changed, strange, and threatening						
15. I find it easy to draw a line between myself and others						
16. My body is a burden to me						
17. My thoughts are flighty and undirected. I find it difficult to think clearly						
18. I am interested in what is happening around me, and it's important to me						
19. My feelings and behaviour are appropriate in the particular situation.						
20. I am full of confidence; everything will be alright.						

Appendix G

		Bilateral					Left						Right					
		Caudate	Insula	Putamen	VS	VTA	Amygdala	Caudate	Insula	Putamen	VS	VTA	Amygdala	Caudate	Insula	Putamen	VS	VTA
Anticipation	High and Low_win_vs_Neutral	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*
	High_win_vs_Neutral	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Low_win_vs_Neutral	*		*	*			*		*	*	*		*		*	*	*
	High_win_vs_Low_win			*	*	*	*			*	*	*	*			*	*	*
Feedback	High_and _low_win_vs_Neutral	*			*		*				*		*	*	*		*	
	High_win_vs_Neutral			*	*			*			*					*		
	Low_win_vs_Neutral								*									
	High_win_vs_Low_win	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	High_win_loss_vs_Neutral	*	*	*	*		*	*	*	*	*		*	*	*	*	*	
	Low_win_loss_vs_Neutral		*	*	*	*	*		*	*	*	*	*		*	*	*	*
	All_win_vs_All_loss	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Appendix H

Research Ethics Office

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Waterloo Road
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Telephone 020 7848 4020/4070/4077
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KING'S College LONDON

3 August 2016

Dear Vasilcia

Reference Number: RESCMR-15/16-0650

Study Title: Rumination as a mechanism of ketamine's antidepressant action: a placebo-controlled acute dose fMRI study.

Modification Review Outcome: Full Approval

Thank you for submitting a modification request for the above study. I am writing to confirm approval of this.

If If you have any questions regarding this application please contact the Research Ethics Office.

Kind regards

Research Ethics Office
on behalf of

PNM Research Ethics Subcommittee